IPW

Office Action Summary	Application No.	Applicant(s)	
	10/566,426	PHILLIPS, JOHN W.	
	Examiner	Art Unit	
	Nina A. Archie	1645	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *30 January 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-11,13,15,17,19-23 and 25-30* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-11,13,15,17,19-23 and 25-30* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *3/23/2007 and 3/3/2008*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Priority

Applicant's claim for domestic priority under 35 U.S.C. 119(e) is acknowledged.

Specification

The lengthy specification has not been checked to the extent necessary to determine the presence of all possible minor errors. Applicant's cooperation is requested in correcting any errors of which applicant may become aware in the specification.

Information Disclosure Statement

The information disclosure statements filed on 3/23/2007 and 3/3/2008 have been considered. Initialed copies are enclosed.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-11, 13, 15, 17, 19, 20-23, and 25-30 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a written description rejection.

The instant claims recite, a method in step (b), with said recitation, "target polynucleotide being a sequence operatively linked to a promoter native to S. cerevisiae gene YMR325W, or a YMR325W promoter sequence homolog comprising one or more

nucleotide substitutions, additions or deletions that do not effect the ability of the sequence to promote transcription of said operatively linked sequence". However, for a promoter to function there must be elements present which guide transcription. While there were many promoters and the elements therefrom known in the art, the promoters of the present invention must be associated with a gene which encodes an enzyme or regulator in the sterol biosynthesis pathway to be functional in a useful way in the invention. The term "homolog" is not defined in the specification, and does not have a precise meaning in the art (see the rejection, below, under 35 USC § 112, second paragraph), and is thus interpreted as reading upon any promoter possessing any degree of similarity to the specifically recited promoters, which thus represents a vast genus. Furthermore, there is no description in the specification of any structural features that would permit any given promoter to function in a relevant way in the present invention. As such, the genus of potential promoter sequences

Furthermore, the instant claims are drawn to a vast genus of homologs thereof of SEQ ID NO: 3. To fulfill the written description requirements set forth under 35 USC § 112, first paragraph, the specification must describe at least a substantial number of the members of the claimed genus, or alternatively describe a representative member of the claimed genus, which shares a particularly defining feature common to at least a substantial number of the members of the claimed genus, which would enable the skilled artisan to immediately recognize and distinguish its members from others, so as to reasonably convey to the skilled artisan that Applicant has possession the claimed invention. To adequately describe the genus of homologs thereof of SEQ ID NO: 3, applicant must also give a functional limitation of homologs thereof of SEQ ID NO: 3.

The specification, however, does not disclose distinguishing and identifying features of a representative member of the genus of homologs thereof of SEQ ID NO: 3 to which the claims are drawn, such as a correlation between structure of the peptide and its recited function, so that the skilled artisan could immediately envision or recognize at least a substantial number of members of the claimed genus of homologs thereof of SEQ ID NO: 3.

MPEP § 2163.02 states, "an objective standard for determining compliance with

the written description requirement is, 'does the description clearly allow persons of ordinary skill in the art to recognize that he or she invented what is claimed". The courts have decided: The purpose of the "written description" requirement is broader than to merely explain how to "make and use"; the applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention is, for purposes of the "written description" inquiry, whatever is now claimed. See Vas-Cath, Inc.'v. Mahurkar, 935 F.2d 1555, 1563-64, 19 USPQ2d 1111, 1117 (Federal Circuit, 1991). Furthermore, the written description provision of 35 USC § 112 is severable from its enablement provision; and adequate written description requires more than a mere statement that it is part of the invention and reference to a potential method for isolating it. See Fiers v. Revel, 25 USPQ2d 1601, 1606 (CAFC 1993)and Amgen Inc. V. Chugai Pharmaceutical Co. Ltd., 18 USPQ2d 1016.

The Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, paragraph 1, "'Written Description" Requirement (66 FR 1099-1111, January 5,2001) state, "[p]ossession may be shown in a variety of ways including description of an actual reduction to practice, or by showing the invention was 'ready for patenting' such as by disclosure of drawings or structural chemical formulas that show that the invention was complete, or by describing distinguishing identifying characteristics sufficient to show that the applicant was in possession of the claimed invention" (Id. at 1104).

The Guidelines further state, "[f]or inventions in an unpredictable art, adequate written description of a genus which embraces widely variant species cannot be achieved by disclosing only one species within the genus" (Id. at 1106); accordingly, it follows that an adequate written description of a genus cannot be achieved in the absence of a disclosure of at least one species within the genus. Bowie et al (Science, 1990, 247:1306-1310) teach that an amino acid sequence encodes a message that determines the shape and function of a protein and that it is the ability of these proteins to fold into unique three-dimensional structures that allows them to function, carry out the instructions of the genome and form immunoepitopes. Bowie et al. further teach that the problem of predicting protein structure from sequence data and in turn utilizing

predicted structural determinations to ascertain functional aspects of the protein is extremely complex. (column 1, page 1306). Bowie et al further teach that while it is known that many amino acid substitutions are possible in any given protein, the position within the protein's sequence where such amino acid substitutions can be made with a reasonable expectation of maintaining function are limited. Certain positions in the sequence are critical to the three dimensional structure/function relationship and these regions can tolerate only conservative substitutions or no substitutions (column 2, page 1306). Therefore, in accordance with the Guidelines, the description of homologs thereof of SEQ ID NO: 3 is not deemed representative of the genus of SEQ ID NO: 3 of the claim invention thus the claim does not meet the written description requirement.

Claim Rejections - 35 USC § 102 and 103

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless -
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

> (e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

The changes made to 35 U.S.C. 102(e) by the American Inventors Protection Act of 1999 (AIPA) and the Intellectual Property and High Technology Technical Amendments Act of 2002 do not apply when the reference is a U.S. patent resulting directly or indirectly from an international application filed before November 29, 2000. Therefore, the prior art date of the reference is determined under 35 U.S.C. 102(e) prior to the amendment by the AIPA (pre-AIPA 35 U.S.C. 102(e)).

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-3, 5-9, 13, 15, 17, 19, 21, 23, 25, and 28-30 rejected under 35 U.S.C. 102(e) as being unpatentable over Dixon et al US Patent No. 6828092 B1 Date December 7, 2004 US Filing Date January 12, 1998.

Examiner interprets homolog comprising one ore more nucleotide subsitutions, addition or deletion to be any promoter sequence.

Claims 1-3, 5-9, 13, 15, 17, 19, 21, 23, 25, and 28-30 are drawn to a method for determining whether a molecule affects the function or activity of a sterol biosynthesis pathway in a S. cerevisiae cell comprising: (a) contacting said cell with, or recombinantly expressing within said cell, said molecule; (b) determining whether RNA expression or protein expression in said cell of a target polynucleotide sequence is changed in step (a) relative to the expression of said target polynucleotide sequence in the absence of said molecule, said target polynucleotide being a sequence operatively linked to a promoter native to S. cerevisiae gene YMR325W, or a YMR325W promoter homolog comprising one or more nucleotide substitutions, additions or deletions that do not effect the ability of the sequence to promote transcription of said operatively linked sequence; and (c)
determining that said molecule affects the function or activity of said sterol biosynthesis pathway if expression of said target polynucleotide is changed, or determining that said molecule does not affect the function or activity of said sterol biosynthesis pathway if expression of said target polynucleotide sequence is unchanged (claim 1); a method for monitoring activity of a sterol biosynthesis pathway in a S. cerevisiae cell exposed to a molecule comprising: (a) contacting said cell with, or recombinantly expressing within said cell, said molecule; (b) determining whether RNA expression or protein expression in said cell of a target polynucleotide sequence is changed in step (a) relative to expression of said target polynucleotide sequence in the absence of said molecule, said target polynucleotide sequence being regulated by a promoter native to a S. cerevisiae YMR325W gene or a YMR325W homolog comprising one or more nucleotide substitutions, additions or deletion that do not effect the ability of the sequence to promote regulated transcription of said target polynucleotide sequence; and (c) determining that the activity of the sterol biosynthesis pathway in said cell is changed if expression of said target polynucleotide is determined to be changed in step (b), or determining that the activity of the sterol biosynthesis pathway in said cell is unchanged if expression of said target polynucleotide is determined to be unchanged in

step (b) (claim 13); a method for identifying a molecule that modulates expression of a sterol biosynthesis pathway target polynucleotide sequence comprising: (a) recombinantly expressing in a S. cerevisiae cell, or contacting a S. cerevisiae cell with, at least one candidate molecule; and (b) measuring RNA or protein expression in said cell of a target polynucleotide sequence, said target polynucleotide sequence being regulated by a promoter native to a S. cerevisiae YMR325W gene or a YMR325W sequence homolog comprising one or more nucleotide substitutions, additions or deletions that do not effect the ability of the sequence to promote regulated transcription of said target polynucleotide sequence thereof ; wherein an increase or decrease in expression of said target polynucleotide sequence relative to expression of said target polynucleotide sequence in the absence of said candidate molecule indicates that said candidate molecule modulates expression of said sterol biosynthesis pathway target polynucleotide sequence (claim 22).

Dixon et al teach a method for the identification of agents which modulate sterol biosynthesis which method comprises contacting a test compound with a host cell of S. cerevisiae comprising a DNA sequence which controls expression of a yeast acetoacetyl CoA thiolase gene operably linked to a reporter system such that modulation of sterol biosynthesis in the host cell leads to a detectable change in cell phenotype, and determining whether any such detectable change has occurred (see abstract). Dixon et al teach one or more individual enzymes from the pathway are selected, and compounds are screened for their ability to inhibit these enzymes. Dixon et al teach that operably linked means linked in such a way as to provide the basic sequence signals necessary for initiation of gene transcription and initiation of gene translation. Dixon et al teach in vivo assays for inhibitors of sterol biosynthesis wherein inhibition leads to a change in the level of expression of a reporter gene and nucleic acids and recombinant cells use in the assays. Dixon et al teach that the activity of the reporter gene when grown under aerobic conditions in the absence of inhibitors of sterol biosynthesis is low. Dixon et al teach that the promoter region of S. cerevisiae acetoacetyl CoA thiolase is linked to a reporter gene the reporter gene may be induced by sterol biosynthesis inhibitors. Dixon et al teach an assay which is capable of

detecting a wide range of inhibitors of sterol biosynthesis and that the assay is simple, cheap and robust and may be employed in high throughput mode to screen large chemical collections, natural product collections and compound libraries. Dixon et al teach that the assay described here may be used in combination with another reporter system in the same cell, allowing for compounds to be simultaneously screened for the ability to modulate sterol biosynthesis and other processes. Dixon et al teach novel forms of green fluorescent proteins with different absorption spectra that allow the use of multifunctional assays in the cell using the same output. Dixon et al teach that the advantage of using a reporter gene as a reporter system is that it confers a readily measurable phenotype upon the cell and that the reporter gene may conveniently comprise the coding sequence of a enzyme such as firefly luciferase, E. coli choramphenicol acetyl transferase, or green fluorescent protein, in which the phenotype conferred may be measured by alterations in fluorescence (see abstract claims.

Thus Dixon et al teach a method for determining whether a molecule affects the function or activity and a method for monitoring activity of a sterol biosynthesis pathway in a S. cerevisiae cell comprising: (a) contacting said cell with, or recombinantly expressing within said cell, said molecule; (b) determining whether RNA expression or protein expression in said cell of a target polynucleotide sequence is changed in step (a) relative to the expression of said target polynucleotide sequence in the absence of said molecule, said target polynucleotide being a sequence operatively linked to a promoter native to S. cerevisiae gene YMR325W promoter homolog comprising one or more nucleotide substitutions, additions or deletions that do not effect the ability of the sequence to promote transcription of said operatively linked sequence thereof ; and (c) determining that said molecule affects the function or activity and a method of determining that the activity of said sterol biosynthesis pathway if expression of said target polynucleotide is changed, or determining that said molecule does not affect the function or activity of said sterol biosynthesis pathway if expression of said target polynucleotide sequence is unchanged, wherein said target polynucleotide sequence comprises a marker gene; wherein step (b) comprises determining whether the RNA expression or protein expression of said marker gene is changed in step (a) relative to

the expression of said marker gene in the absence of the molecule; and wherein step (c) comprises determining that said molecule affects the function or activity of said sterol biosynthesis pathway if expression of said marker gene is changed, or determining that said molecule does not affect the function or activity of said sterol biosynthesis pathway if expression of said marker gene is unchanged, wherein said molecule inhibits sterol biosynthesis such that said cell contacted with the molecule exhibits a lower level of sterol than a second cell which is not contacted with said molecule, wherein step (b) comprises determining whether RNA expression is changed, wherein step (b) comprises determining whether protein expression is changed, wherein step (c) comprises determining that said molecule inhibits sterol biosynthesis if expression of said target polynucleotide sequence in step (a) is increased relative to expression of said target polynucleotide sequence in the absence of said molecule, wherein the S. cerevisiae cell is a cell that recombinantly expresses said target polynucleotide sequence, wherein step (a) comprises contacting said cell with said molecule, and wherein step (a) is carried out in a liquid high throughput-like assay, wherein said molecule are proteins.

Thus Dixon et al teach a method for identifying a molecule that modulates expression of a sterol biosynthesis pathway target polynucleotide sequence comprising: (a) recombinantly expressing in a S. cerevisiae cell, or contacting a S. cerevisiae cell with, at least one candidate molecule; and (b) measuring RNA or protein expression in said cell of a target polynucleotide sequence, said target polynucleotide sequence being regulated by a YMR325W sequence homolog comprising one or more nucleotide substitutions, additions or deletions that do not effect the ability of the sequence to promote regulated transcription of said target polynucleotide sequence; wherein an increase or decrease in expression of said target polynucleotide sequence relative to expression of said target polynucleotide sequence in the absence of said candidate molecule indicates that said candidate molecule modulates expression of said sterol biosynthesis pathway target polynucleotide sequence, wherein step (a) comprises contacting said cell with a second, test cell, wherein said test cell produces said molecule, wherein said molecule is released by said test cell, wherein said

molecule is secreted by said test cell (see abstract, claims, columns 1-2 and 5-10 and example 1).

Claims 1-11, 13, 15, 17, 19-23, and 25-30 rejected under 35 U.S.C. 103(a) as being unpatentable over Dixon et al US Patent No. 6828092 B1 Date December 7, 2004 US Filing Date January 12, 1998 in view of Ashby et al WO/2000/58521 Date October 5, 2000, Phillips, J. US Patent No: 7022481B2 Date April 4, 2006 US Filing Date December 19, 2002, and Contreras et al. WO/2001/02550A2 Date January 11, 2001.

Claims 1-11, 13, 15, 17, 19-23, and 25-30 are drawn to A method for determining whether a molecule affects the function or activity of a sterol biosynthesis pathway in a S. cerevisiae cell comprising: (a) contacting said cell with, or recombinantly expressing within said cell, said molecule; (b) determining whether RNA expression or protein expression in said cell of a target polynucleotide sequence is changed in step (a) relative to the expression of said target polynucleotide sequence in the absence of said molecule, said target polynucleotide being a sequence operatively linked to a promoter native to S. cerevisiae gene YMR325W, or a YMR325W promoter homolog comprising one or more nucleotide substitutions, additions or deletions that do not effect the ability of the sequence to promote transcription of said operatively linked sequence; and (c) determining that said molecule affects the function or activity of said sterol biosynthesis pathway if expression of said target polynucleotide is changed, or determining that said molecule does not affect the function or activity of said sterol biosynthesis pathway if expression of said target polynucleotide sequence is unchanged (claim 1); a method for monitoring activity of a sterol biosynthesis pathway in a S. cerevisiae cell exposed to a molecule comprising: (a) contacting said cell with, or recombinantly expressing within said cell, said molecule; (b) determining whether RNA expression or protein expression in said cell of a target polynucleotide sequence is changed in step (a) relative to expression of said target polynucleotide sequence in the absence of said molecule, said target polynucleotide sequence being regulated by a promoter native to a S. cerevisiae YMR325W gene or a YMR325W homolog comprising one or more nucleotide substitutions, additions or deletion that do not effect the ability of the sequence to

promote regulated transcription of said target polynucleotide sequence; and (c) determining that the activity of the sterol biosynthesis pathway in said cell is changed if expression of said target polynucleotide is determined to be changed in step (b), or determining that the activity of the sterol biosynthesis pathway in said cell is unchanged if expression of said target polynucleotide is determined to be unchanged in step (b) (claim 13); a method for identifying a molecule that modulates expression of a sterol biosynthesis pathway target polynucleotide sequence comprising: (a) recombinantly expressing in a S. cerevisiae cell, or contacting a S. cerevisiae cell with, at least one candidate molecule; and (b) measuring RNA or protein expression in said cell of a target polynucleotide sequence, said target polynucleotide sequence being regulated by a promoter native to a S. cerevisiae YMR325W gene or a YMR325W sequence homolog comprising one or more nucleotide substitutions, additions or deletions that do not effect the ability of the sequence to promote regulated transcription of said target polynucleotide sequence thereof ; wherein an increase or decrease in expression of said target polynucleotide sequence relative to expression of said target polynucleotide sequence in the absence of said candidate molecule indicates that said candidate molecule modulates expression of said sterol biosynthesis pathway target polynucleotide sequence (claim 22).

Dixon is relied upon as set forth supra. However Dixon et al does not teach a method using YMR325W promoter, Dixon et al does not teach a method, wherein step (a) comprises contacting said cell with said molecule, and wherein step (a) is carried out in a solid plate halo assay, wherein step (a) comprises contacting said cell with said molecule, and wherein step (a) is carried out in an agar overlay assay, wherein said molecule is purified, wherein said molecule is not substantially purified, wherein said promoter comprises SEQ ID NO: 3 or a SE0 ID NO: 3 homolog comprising one or more nucleotide substitutions, additions or deletions that do not effect the ability of the sequence to promote transcription of said operatively linked sequence thereof.

Ashby teach methods of identifying genes whose expression is indicative of activation of a particular biochemical or metabolic pathway or a common set of biological reactions or functions in a cell ("regulon indicator genes"). Ashby et al teach

methods for identifying effectors (activators and inhibitors) of regulon target genes are provided. Ashby et al teach genes that are regulated by regulon target genes of yeast or its mammalian homolog may be identified comprising the steps of a) overexpressing the target gene in host cells of a matrix comprising a plurality of units of cells, the cells in each unit containing a reporter gene operably linked to an expression control sequence derived from a gene of a selected organism; and b) identifying genes that are either induced or repressed by overexpression of the target gene. Ashby et al teach yeast cells respond by significantly up-regulating the genes encoding sterol biosynthetic enzymes and thus synthesizing more of the enzymes that make sterols and identifying genes that are involved in sterol biosynthesis or in related metabolic pathways by assays (see abstract claims, pgs. 1 and 15-22).

Ashby et al teach an isolated protein or polypeptide that has been separated from naturally associated components that accompany it in its native state. Thus, a polypeptide that is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be "isolated" from its naturally associated components. Ashby et al teach a protein may also be rendered substantially free of naturally associated components by isolation, using protein purification techniques well known in the art. Ashby et al teach a monomeric protein is "substantially pure, "substantially homogeneous" or "substantially purified" when at least about 60 to 75% of a sample exhibits a single polypeptide sequence. Ashby et al teach a substantially pure protein will typically comprise about 60 to 90% W/W of a protein sample, more usually about 95%, and preferably will be over 99% pure. Ashby et al teach a protein purity or homogeneity may be indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualizing a single polypeptide band upon staining the gel with a stain well known in the art and for certain purposes, higher resolution may be provided by using HPLC or other means well known in the art for purification. Ashby et al teach nucleic acids of this invention include single-stranded and double-stranded DNA, RNA, oligonucleotides, antisense molecules, or hybrids thereof and may be isolated from biological sources or synthesized chemically or by recombinant DNA methodology.

Ashby et al teach that the nucleic acids, recombinant DNA molecules and vectors of this invention may be present in transformed or transfected cells, cell lysates, or in partially purified or substantially pure forms (see abstract claims, pgs. 1 and 15-22).

Ashby et al teach S. cerevisiae proteins that have homology to a protein from another organism if the encoded amino acid sequence of the yeast protein has a similar sequence to the encoded amino acid sequence of a protein of a different of a different organism. Ashby et al teach a S. cerevisiae protein may have homology or be homologous to another S. cerevisiae protein if the two proteins have similar amino acid sequences (see abstract claims, pgs. 1 and 15-22). Thus teaching a method, wherein said molecule is purified, wherein said molecule is not substantially purified.

Phillips teach a method in a pathway in S. cerevisiae as set forth supra, wherein step (a) comprises contacting said cell with said molecule, and wherein step (a) is carried out in a solid plate halo assay, wherein step (a) comprises contacting said cell with said molecule, and wherein step (a) is carried out in an agar overlay assay (see claims).

Contreras et al. teach a sequence that is 98% identical to SEQ ID NO:3 thus teaching a YMR325W sequence homolog comprising one or more nucleotide substitutions, additions or deletions that do not effect the ability of the sequence to promote regulated transcription of said target polynucleotide sequence thereof (see claim 1 figure 1 STIC results).

It would have been prima facie obvious at the time the invention was made to incorporate a YMR325W sequence homolog comprising one or more nucleotide substitutions, additions or deletions that do not effect the ability of the sequence to promote regulated transcription of said target polynucleotide sequence thereof as taught by Contreras et al because Contreras et al teach protein and coding sequences of apoptosis associated proteins from the yeast Saccharomyces cerevisiae that can be used to identify treatments for yeast infections (see abstract). It would have been prima facie obvious at the time the invention was made to incorporate a method in a pathway in S. cerevisiae as set forth supra, wherein step (a) comprises contacting said cell with said molecule, and wherein step (a) is carried out in a solid plate halo assay, wherein

step (a) comprises contacting said cell with said molecule, and wherein step (a) is carried out in an agar overlay assay as taught by Phillips J. because both Phillips and Dixon et al teach the same method of determining the function, monitoring, and identifying a molecule in a biosynthesis pathway of S. cerevisiae. It would have been prima facie obvious at the time the invention was made to incorporate a method, wherein said molecule is purified, wherein said molecule is not substantially purified as taught by Ashby et al because both Ashby et al and Dixon et al teach the same method of determining the function, monitoring, and identifying a molecule in a biosynthesis pathway of S. cerevisiae.

Status of the Claims

Claims 1-11, 13, 15, 17, 19-23, and 25-30 are rejected.

No claims are allowed.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Nina A. Archie whose telephone number is 571-272-9938. The examiner can normally be reached on Monday-Friday 8:30-5:00p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner supervisor, Shanon Foley can be reached on 571-272-0898. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Nina Archie
Examiner
Art Unit 1645

/Nina A Archie/
Examiner, Art Unit 1645
/N. A. A./
Examiner, Art Unit 1645

/Mark Navarro/
Primary Examiner, Art Unit 1645

Notice of References Cited	Application/Control No. 10/566,426	Applicant(s)/Patent Under Reexamination PHILLIPS, JOHN W.	
	Examiner Nina A. Archie	Art Unit 1645	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-6,828,092	12-2004	Dixon et al.	435/6
*	B	US-7,022,481	04-2006	Phillips, John W.	435/6
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N	WO/2000/58521	10-2000	US	Ashby et al	
	O	WO/2001/02250	01-2001	BE	Contreras et al.	
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Bowie et al (Science, 1990, 247:1306-1310
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

PATENT Case No. RS0212

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: John W. Phillips

Serial No. 10/566,426

Filed: January 30, 2006

For: Methods for Using a Sterol Biosynthesis Pathway Reporter Gene to Screen for Antifungal or Lipid Lowering Compounds

Art Unit: _____

Examiner: To Be Assigned

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

INFORMATION DISCLOSURE STATEMENT UNDER 37 CFR 1.97

Sir:

1. In compliance with 37 C.F.R. 1.97, submitted on the attached form herewith is a list of patents, publications or other information which are requested to be made of record in this application. This Information Disclosure Statement is not an admission that any patent, publication or other information referred to herein is "prior art" for this invention. In accordance with 37 C.F.R. 1.97(h), the filing of this Information Disclosure Statement shall not be construed to be an admission that the information cited in the Statement is, or is considered to be, material to patentability as defined in 37 C.F.R. 1.56(b).

2. In accordance with 37 C.F.R. 1.97(g), the filing of this Information Disclosure Statement shall not be construed to mean that a search has been made.

3. Applicants respectfully request that the Examiner initial the attached form after reviewing the pertinence of each reference.

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ROSETTA INPHARMATICS LLC

By R. Douglas Brallier Date 20 March 2007

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INFORMATION DISCLOSURE STATEMENT

5. Pursuant to 37 C.F.R. 1.98(d), copies of references listed on the attached form that were submitted to or cited by the Office in a related application upon which the instant application relies for an earlier filing date under 35 U.S.C. 120 are not enclosed. Related application(s) in which references were submitted to or cited by the Office are as follows:

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Respectfully submitted,

R. Douglas Bradley

By: R. Douglas Bradley
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Date: March 20, 2007

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Sheet 1 of 4

COMPLETE IF KNOWN	
Application Number	10/566,426
Filing Date	January 30, 2006
First Named Inventor	Phillips
Group Art Unit	
Examiner Name	To Be Assigned
Attorney Docket Number	RS0212

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.	U.S. Patent Document Number	Kind Code (if known)	Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY
	A1	5,569,588		Ashby et al.	10/29/1996
	A2	5,777,888		Rine et al.	07/07/1998
	A3	5,965,352		Stoughton et al.	10/12/1999
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	A8	6,271,002		Lindsley et al.	08/07/2001
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FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.	Foreign Patent Document Office	Number	Kind Code (if known)	Name of Patentee or Applicant of Cited Document	Date of Publication of Cited Document MM-DD-YYYY
	B1	PCT	WO 98/38329		Friend et al.	09/03/1998
	B2	PCT	WO 00/58521		Ashby et al.	10/05/2000

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Application Number	10/566,426
Filing Date	January 30, 2006
First Named Inventor	Phillips
Group Art Unit	
Examiner Name	To Be Assigned
Attorney Docket Number	RS0212

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Examiner Initials*	Cite No.	Include name of the author, title, date, page(s), volume-issue number(s) and place of publication.
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Sheet	3	of	4

COMPLETE IF KNOWN	
Application Number	10/566,426
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First Named Inventor	Phillips
Group Art Unit	
Examiner Name	To Be Assigned
Attorney Docket Number	RS0212

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NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.	Include name of the author, title, date, page(s), volume-issue number(s) and place of publication.
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Examiner Signature	/Nina Archie/	Date Considered	06/17/2008

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NON PATENT LITERATURE DOCUMENTS

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/N.A./		International Search Report for PCT/US2004/24034, Dated January 13, 2005 (3 pages)
/N.A./		Written Opinion of the International Searching Authority for PCT/US2004/24034 (3 pages)

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(54) Title: METHODS FOR THE IDENTIFICATION OF REPORTER AND TARGET MOLECULES USING COMPREHENSIVE GENE EXPRESSION PROFILES

(57) Abstract

The present invention relates to methods of identifying genes whose expression is indicative of activation of a particular biochemical or metabolic pathway or a common set of biological reactions or functions in a cell ("regulon indicator genes"). The present invention provides an example of such an indicator gene. The present invention also relates to methods of partially characterizing a gene of unknown function by determining which biological pathways, reactions or functions its expression is associated with, thereby placing the gene within a functional genetic group or "regulon". These partially characterized genes may be used to identify desirable therapeutic targets of biological pathways of interest ("regulon target genes"). The present invention provides examples of such target genes. Methods for identifying effectors (activators and inhibitors) of regulon target genes are provided. The present invention also provides examples of regulon target gene inhibitors.

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Methods for the Identification of Reporter and Target Molecules Using Comprehensive Gene Expression Profiles

TECHNICAL FIELD OF THE INVENTION

The present invention relates to methods of identifying genes whose expression is indicative of activation of a particular biochemical or metabolic pathway or a common set of biological reactions or functions in a cell ("regulon indicator genes"). The present invention provides an example of such an indicator gene. The present invention also relates to methods of partially characterizing a gene of unknown function by determining which biological pathways, reactions or functions its expression is associated with, thereby placing the gene within a functional genetic group or "regulon". These partially characterized genes may be used to identify desirable therapeutic targets of biological pathways of interest ("regulon target genes"). The present invention provides examples of such target genes. Methods for identifying effectors (activators and inhibitors) of regulon target genes are provided. The present invention also provides examples of regulon target gene inhibitors.

BACKGROUND OF THE INVENTION

The sequencing of the *S. cerevisiae* genome marked the first complete, ordered set of genes from a eukaryotic organism, and revealed the presence of over 6,000 genes on 16 chromosomes (Mewes et al., 1997, Goffeau et al., 1996). The DNA sequence revealed the presence of 6275 known and hypothetical open reading frames (ORFs) encoding putative proteins longer than 99 amino acids in length. Based upon codon usage, which can serve as a predictor of whether or not an ORF is actually expressed, there are currently thought to be 6222 expressed ORFs (Cherry et al., 1997).

The sequence of the roughly 6,000 ORFs in the yeast genome is compiled in the Saccharomyces Genome Database (SGD). The SGD provides Internet access to the complete genomic sequence of *S. cerevisiae*, ORFs, and the putative polypeptides encoded by these ORFs. The SGD can be accessed via the World Wide Web at http://genome-www.stanford.edu/Saccharomyces/ and http://www.mips.biochem.mpg.de/mips/yeast/. A gazetteer and genetic and physical maps of *S. cerevisiae* is found in Mewes et al., 1997 (incorporated herein by reference). References therein also contain the sequence of each chromosome of *S. cerevisiae* (incorporated herein by reference).

Having the complete DNA sequence of yeast available creates an opportunity to take a collectivist, rather than a reductionist, view on biology. We have developed a new technology that enables the simultaneous measurement of gene expression across an entire genome. The Genome Reporter Matrix™ (GRM) is a matrix of units comprising living yeast cells, the cells in each unit containing one yeast reporter fusion (GRM construct) representative of essentially every known hypothetical ORF of *S. cerevisiae*. See U.S. Pat. Nos. 5,569,588 and 5,777,888. A GRM construct comprises the promoter, 5' upstream untranslated region and usually the first four amino acids from one of each hypothetical ORF fused to a gene encoding an easily assayed reporter, such as green fluorescent protein (GFP), luciferin, or β-galactosidase. For a few GRM constructs, one to ten of the first amino acids from a hypothetical ORF is fused to the reporter. In addition, for those ORFs that have an intron, the entire first exon and the usually first four amino acids of the second exon are fused to the reporter. The GRM constructs are able to reveal changes in transcription for each hypothetical ORF in response to specific stimuli. In addition, the GRM constructs are able to reveal changes in mRNA splicing, translation and protein stability in those cases in which the N-terminus of the protein is sufficient for regulation.

The GRM provides an unprecedented view into the compensatory changes a cell makes in the face of a changing environment. Such environmental changes may be in the form of pH, salinity, temperature, osmotic pressure, nutrient

availability, as well as biochemical perturbations caused by xenobiotics, pharmaceutical compounds and mutation. Identifying the compensatory changes a cell makes in response to exposure to a chemical can provide insight into the biological target of the chemical. For example, treatment of the GRM with the cholesterol-lowering drug lovastatin causes the cells to become depleted for sterols and non-sterol isoprenoids. The yeast cells respond by significantly up-regulating the genes encoding sterol biosynthetic enzymes and thus synthesizing more of the enzymes that make sterols. One may identify those genes that are involved in sterol biosynthesis or in related metabolic pathways by assaying the GRM. Because natural selection operates on a selected outcome rather than on a particular molecular mechanism, gene expression profiling strategies that detect regulatory changes through several molecular mechanisms contribute to a fuller view of how regulatory circuits have evolved.

An understanding of the regulatory circuits of yeast serves two purposes. On the one hand, yeast is an ideal model system for eukaryotic cells, including mammalian cells. Therefore, an understanding of the metabolic pathways of yeast can be used to design or discover drugs for use in plants and animals, including humans. On the other hand, yeast possess certain metabolic pathways and genes which are unique to yeast. An understanding of the differences between yeast and higher eukaryotes will permit the design and discovery of antifungal drugs that target genes and metabolic pathways specific to yeast. See U.S. Serial No. 60/127,272, filed concurrently herewith.

Yeast cells are eukaryotic and have many pathways that are similar or identical to those of mammalian cells. However, because yeast cells are unicellular, they are easier to manipulate experimentally and the results of such manipulations are easier to determine. Thus, yeast serves as an ideal model system for eukaryotic cells, including mammalian cells. The deduced protein sequences of the yeast genome display a significant amount of sequence identity with mammalian proteins. About one-third of the yeast ORFs, when aligned with their mammalian counterparts, produce a P-value score of less than 1×10^{-10} (Botstein et al., 1997). This number may in fact be a significant underestimate because the alignments were done with GenBank entries

that make up only about 10-20% of the unique human protein sequences thought to exist.

The evolutionary conservation between yeast and humans is not limited to sequence identity. The list of human genes that can functionally substitute for their yeast counterparts is extensive. For example, H-Ras (Kataoka et al., 1985), HMG-CoA reductase (Basson et al., 1988) and the heme A:farnesyltransferase (Glerum and Tzagoloff, 1994) have been shown to functionally replace their yeast counterparts. Researchers have utilized this evolutionary conservation to clone mammalian genes through their ability to complement the corresponding yeast mutants. Two examples include *CDC2* (Lee and Nurse, 1987) and *CDK2* (Elledge and Spottswood, 1991).

Functional conservation between yeast and humans may be best illustrated by the notable lack of antifungal therapeutic agents available for safely treating systemic infections in humans. Antifungal agents certainly exist, but they are characterized by profound side effects likely caused by inhibition of the mammalian counterparts of the yeast target. L659,699, lovastatin, and zaragozic acid inhibit different steps in the yeast sterol pathway (HMG-CoA synthase, HMG-CoA reductase, and squalene synthase, respectively). These inhibitors are also potent inhibitors of the corresponding mammalian enzymes (Correll and Edwards, 1994). In addition, we have found that in experiments with over 100 pharmaceutical agents used to treat a variety of distinct clinical indications in mammals, approximately 80% produced significant changes in gene expression in the GRM, indicating that there is substantial overlap in drug specificity between mammalian and yeast systems.

Yeast also contain genes that encode proteins that do not have plant and/or animal homologs. These non-homologous genes may be used as targets for the design and discovery of highly specific antifungal agents for use in plants and animals, including humans. The GRM may be used to identify genes that are expressed in particular metabolic pathways. Non-homologous genes in a pathway of interest may be used as targets for design and discovery of antifungal agents, for instance. See, e.g., U.S. Serial No. 60/127,272, filed concurrently herewith.

One metabolic pathway of interest for identification of both

homologous and non-homologous genes is the pathway for synthesis of isoprenoids. Eukaryotic cells utilize a group of structurally related compounds, the isoprenoids, for a vast array of cellular processes. These processes include structural composition of the lipid bilayer, electron transport during respiration, protein glycosylation, tRNA modification, and protein prenylation. All isoprenoids are synthesized via a pathway known variously as the isoprenoid pathway, mevalonate pathway, or sterol biosynthetic pathway. Although the bulk end product of the pathway is sterols, there are several branches of the pathway that lead to non-sterol isoprenoids. Due to the involvement of isoprenoids in a variety of physiologically and medically important processes, a comprehensive understanding of the regulation of this pathway would offer many scientific and practical benefits.

The regulation of the isoprenoid biosynthetic pathway is known to be complex in all eukaryotic organisms examined, including *S. cerevisiae*. The overriding principle for the regulation of this pathway is multiple levels of feedback inhibition. This feedback regulation is keyed to multiple intermediates and appears to act at numerous steps of the pathway, involving changes in transcription, translation and protein stability. Additionally, the availability of molecular oxygen, required for sterol and heme biosynthesis, also regulates the expression of genes at key steps of the pathway. The emerging picture is that the isoprenoid pathway has numerous points of regulation that act to control overall flux through the pathway as well as the relative flux through various branches of the pathway.

Given the complexity of the isoprenoid pathway, it can be difficult to understand the regulation of any one step of this pathway, unless it is viewed within the context of the entire pathway. Thus, the GRM is ideal for understanding the regulation of the isoprenoid pathway because one may observe the regulation of all the yeast genes involved in the isoprenoid pathway at one time by using the GRM. In addition, analysis of the gene expression provided by the GRM (preferably using software described below) may provide information about which particular genes in the isoprenoid pathway are important regulatory genes in the pathway, those which are important indicator genes of the isoprenoid pathway, and those which are suitable

targets to regulate isoprenoid synthesis.

Today we have the luxury of reflecting upon the wealth of information that has come from decades of research into the cell biology and genetics of yeast. Still, less than 20% of the hypothetical ORFs discovered by the yeast genome project had been previously identified through basic research (Goffeau et al., 1996). Additionally, 25% of the yeast ORFs with obvious human homologs have no known function (Botstein et al., 1997). The situation will likely be the same when the human genome sequence is completed.

Several research groups have created software programs that enable the comparison of both chemical and genetic expression profiles to identify related gene expression response patterns, as shown, for example, in Figure 38. In addition, expression changes of individual genes in response to any given treatment can often be accessed through hypertext links. Currently, our software will: 1) normalize expression data; 2) rank changes in individual gene's expression relative to a particular treatment; 3) rank similarities between genomic expression profiles as a result of a chemical or genetic treatment; and 4) determine the correlation coefficient for an individual gene's expression relative to that of all other genes to identify regulons, or groups of genes that share the same regulatory programs. See United States Application 09/076,668, now pending; Eisen et al. (1998); and Tamayo et al. (1999).

The ability to assign ORFs to functional groups based upon their expression patterns will provide valuable information pertaining to the function of proteins from model organisms as well as their mammalian counterparts. Analysis of genomic expression patterns may also reveal upstream regulatory sequences, including promoters, with great utility for regulated or constituitive expression of recombinant genes. Such regulated sequences can be used for making reporter constructs for any selected process intrinsic to a given genome.

These functional genomics studies will provide a great deal of information that can implicate yeast genes, as well as their mammalian counterparts, in a variety of cellular functions. Associations of particular genes with specific biological pathways will be made by virtue of the genes' patterns of regulation under numerous

conditions.

One particular problem in the prior art has been identifying genes whose expression is representative of a specific biological (e.g., metabolic) pathway. One would like to be able to measure the expression of a gene or its encoded protein to indicate the effect of a particular treatment on a specific pathway. Thus, there is a need for various pathway indicator genes for the various metabolic pathways.

A second problem in the prior art has been identifying genes and their encoded proteins which can be efficient targets within a specific biochemical pathway or set of associated pathways. Once good targets have been identified, pharmaceutical compounds and treatments may be designed or discovered to regulate the expression or activity of the target gene or protein.

SUMMARY OF THE INVENTION

The instant invention addresses the above problems by providing a method using genomic arrays, such as the GRM or hybridization arrays, for identifying indicator genes that are specific for particular biochemical pathways and sensitive to perturbations of these pathways. The instant invention provides one such gene, *HES1*, which is an indicator for the isoprenoid metabolic pathway. The invention provides the polynucleotide sequence of *HES1* and vectors and host cells comprising this sequence. The invention also provides a method of producing *HES1* recombinantly. The invention further provides methods of using *HES1* as a specific indicator of the state of the isoprenoid pathway to identify compounds that regulate that pathway.

The instant invention also provides a method for identifying targets for one or more biochemical pathways of interest using the GRM or other types of genomic arrays, such as hybridization arrays. The instant invention also provides a number of ORFs and their encoded proteins which are targets for lipid metabolism, yeast morphology, RNA metabolism and growth control. These ORFs include *YMR134w*, *YER034w*, *YJL105w*, *YKL077w*, *YGR046w*, *YJR041c*, *YER044c* and *YLR100w* and their encoded proteins.

The invention provides the polynucleotide sequences of these ORFs and

vectors and host cells comprising these ORFs for use in methods of identifying, designing and discovering highly specific anti-target agents. Specific anti-target agents include antisense nucleic acid molecules that target *YMR134w*, *YER034w*, *YJL105w*, *YKL077w*, *YGR046w*, *YJR041c*, *YER044c* and *YLR100w* and ribozymes that cleave RNAs encoded by these ORFs. The invention also provides a methods of recombinantly producing the protein encoded by these ORFs for use as a target in methods of identifying, designing and discovering highly specific antifungal agents and for producing antibodies directed against the encoded protein. Specific anti-target agents include antibodies that bind to the protein encoded by *YMR134w*, *YER034w*, *YJL105w*, *YKL077w*, *YGR046w*, *YJR041c*, *YER044c* and *YLR100w* and small organic molecules that bind to and inhibit proteins encoded by these ORFs.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Summary of Characteristics for *YJL105w*.

Figure 2. Plot of changes in expression of *YJL105w* and *CYB5* in response to different chemical treatments. Each point represents the expression changes in a given chemical treatment. The fitness of the points to a line provides an indication of the level of coordinate gene expression. *CYB5* functions in sterol biosynthesis through its activation of the Erg11p NADPH-cytochrome P-450 reductase.

Figure 3. Regulated Expression of *YJL105w*. *YJL105w* is significantly induced by isoprenoid biosynthetic inhibitors and mutations in HMG-CoA synthase (hmgs). "Log Ratio" refers to the natural log ratio of treated/untreated expression values.

Figure 4. Effects of lovastatin on wild-type and YJL105w knockout yeast strains. 10 µl of a 25 mg/ml solution of lovastatin (250 µg) in ethanol was applied to a sterile drug disk on a lawn of yeast (5×10^6 cells, ABY363). The plates were incubated overnight at 30°C.

Figure 5. Summary of Characteristics for *YMR134w*.

Figure 6. Plot of changes in expression of *YMR134w* and *ERG2* in

response to different chemical treatments. Each point represents the expression changes in a given chemical treatment. The fitness of the points to a line provides an indication of the level of coordinate gene expression. *ERG2* encodes sterol isomerase.

Figure 7. Treatments Causing Highest Expression of *YMR134w*. *YMR134w* is induced most significantly by inhibitors of the isprenoid biosynthetic pathway.

Figure 8. Database Searches with *YMR134w*. Database searches with *YMR134w* did not reveal any apparent mammalian counterparts.

Figure 9. Summary of Characteristics for *YER044c*.

Figure 10. Plot of changes in expression of *YER044c* and *ERG2* in response to different chemical treatments. Each point represents the expression changes in a given chemical treatment. The fitness of the points to a line provides an indication of the level of coordinate gene expression.

Figure 11. Treatments Causing Highest Expression of *YER044c*. *YER044c* is induced most significantly by inhibitors of the isprenoid biosynthetic pathway.

Figure 12. Database Searches with *YER044c*. Database searches with *YER044c* reveal numerous mammalian expressed-sequence tag (EST) apparent counterparts.

Figure 13. Comparison of the *YER044c* Predicted Protein Sequence with Mouse and Human EST Translations.

Figure 14. Comparison of the *YER044c* Predicted Protein Sequence with Rat EST Translation.

Figure 15. Summary of Characteristics for *YLR100w*.

Figure 16. Plot of changes in expression of *YLR100w* and *CYB5* in response to different chemical treatments. Each point represents the expression changes in a given chemical treatment. The fitness of the points to a line provides an indication of the level of coordinate gene expression.

Figure 17. Treatments Causing Highest Expression of *YLR100w*. *YLR100w* is induced most significantly by inhibitors of isprenoid biosynthesis and a

mutation in the gene encoding Erg11p.

Figure 18. Database Searches with *YLR100w*. Database searches with *YLR100w* reveal numerous mammalian expressed-sequence tag (EST) apparent counterparts.

Figure 19. Alignment of *YLR100w* to Mammalian ESTs.

Figure 20. Summary of Characteristics for *YER034w*.

Figure 21. Plot of changes in expression of *YER034w* and *GPA2* in response to different chemical treatments. Each point represents the expression changes in a given chemical treatment. The fitness of the points to a line provides an indication of the level of coordinate gene expression. Gpa2p, encoded by *GPA2*, is the alpha subunit of a trimer G-protein involved in pseudohyphal growth.

Figure 22. Mutation of the *YER034w* Gene Leads to Increased Pseudohyphal Growth. Cells were plated onto low nitrogen plates (0.5% agarose, 2% glucose, 0.34% yeast nitrogen base without amino acids and ammonium sulfate, 0.05mM ammonium sulfate, 20 μg/ml uracil, 30 μg/ml leucine, and 5 μg/ml histidine) and incubated for four days at 25°C. Bar height represents the average number of hyphal projections per colony (n=20).

Figure 23. Summary of Characteristics for *YKL077w*.

Figure 24. Plot of changes in expression of *YKL077w* and *SGV1* in response to different chemical treatments. Each point represents the expression changes in a given chemical treatment. The fitness of the points to a line provides an indication of the level of coordinate gene expression. *SGV1* is a Cdc28p-related protein kinase that is essential for yeast viability.

Figure 25. Expression Correlation of *YKL077w*. Expression of the *YKL077w* gene correlates with that of genes involved in cell wall integrity and cytoskeletal reorganization.

Figure 26. Database Searches with *YKL077w*. Database searches with *YKL077w* did not reveal any apparent mammalian counterparts.

Figure 27. Summary of Characteristics for *YGR046w*.

Figure 28. Plot of changes in expression of *YGR046w* and *IRA2* in

response to different chemical treatments. Each point represents the expression changes in a given chemical treatment. The fitness of the points to a line provides an indication of the level of coordinate gene expression. *IRA2* encodes a GTPase-activating protein for Ras1p and Ras2p.

Figure 29. Expression Correlation of *YGR046w*. Expression of the *YGR046w* gene is correlated to other genes involved in growth control.

Figure 30. Treatments Causing the Most Significant Changes in Expression of *YGR046w*. Expression of *YGR046w* is sensitive to agents that perturb mitrochondrial function, create oxidative stress and disrupt the cytoskeleton.

Figure 31. Summary of Characteristics for *YJR041c*.

Figure 32. Plot of changes in expression of *YJR041c* and *MED7* in response to different chemical treatments. Each point represents the expression changes in a given chemical treatment. The fitness of the points to a line provides an indication of the level of coordinate gene expression. *MED7* is a component of the mediator complex involved in RNA Polymerase II transcription.

Figure 33. Expression Correlation of *YJR041c*. Expression of *YJR041c* is correlated to genes involved in RNA metabolism including RNA polymerase I and II transcription, mRNA splicing and turnover and ribosome function.

Figure 34. Database Searches with *YJR041c*. Database searches with *YJR041c* did not reveal any apparent mammalian counterparts.

Figure 35. Summary of Characteristics for *HES1*.

Figure 36. Expression Correlation of *HES1*.

Figure 37. Treatments that Induce the *HES1* Reporter. Inhibitors of the isoprenoid biosynthetic pathway cause a significant induction of the *HES1* reporter.

Figure 38. Browser Interface of Acacia's Expression Software.

Figure 39. *YJL105w* DNA Sequence.

Figure 40. *YJL105w* Protein Sequence.

Figure 41. *YMR134w* DNA Sequence.

Figure 42. *YMR134w* Protein Sequence.

Figure 43. *YER044c* DNA Sequence.

Figure 44. *YER044c* Protein Sequence.

Figure 45. Mouse EST with Similarity to *YER044c*.

Figure 46. Human EST with Similarity to *YER044c*.

Figure 47. Rat EST with Similarity to *YER044c*.

Figure 48. *YLR100w* DNA Sequence.

Figure 49. *YLR100w* Protein Sequence.

Figure 50. Human EST with Similarity to *YLR100w*.

Figure 51. Mouse EST with Similarity to *YLR100w*.

Figure 52. Mouse EST with Similarity to *YLR100w*.

Figure 53. Mouse Gene with Similarity to *YLR100w*.

Figure 54. *YER034w* DNA Sequence.

Figure 55. *YER034w* Protein Sequence.

Figure 56. *YKL077w* DNA Sequence.

Figure 57. *YKL077w* Protein Sequence.

Figure 58. *YGR046w* DNA Sequence.

Figure 59. *YGR046w* Protein Sequence.

Figure 60. *YJR041c* DNA Sequence.

Figure 61. *YJR041c* Protein Sequence.

Figure 62. *HES1* DNA Sequence.

Figure 63. *HES1* Protein Sequence.

Figure 64. Reproducibility of the Genome Reporter Matrix™. Fluorescence from 864 independent untreated reporter-harboring yeast strains was plotted against the corresponding clones of an independent control array.

Figure 65. Rat Gene with Similarity to YLR100w.

Figure 66. *DAK1* DNA Sequence.

Figure 67. *DAK1* Protein Sequence.

Figure 68. *PGU1* DNA Sequence.

Figure 69. *PGU1* Protein Sequence.

Figure 70. *STE18* DNA Sequence.

Figure 71. *STE18* Protein Sequence.

Figure 72. *YGL198w* DNA Sequence.

Figure 73. *YGL198w* Protein Sequence.

Figure 74. Each dot on the 4-quadrant plot represents a treatment affecting the reporters affecting *DAK1* and *PGU1*. Treatments are plotted as to whether *DAK1* was up-regulated (above x-axis) or down-regulated (below x-axis) and whether *PGU1* was up-regulated (right of the y-axis) or down-regulated (left of the y-axis). Thus, conditions where both reporters are up-regulated are in the upper right quadrant. Each division on the graph represents one natural log ratio change relative to controls. The *hog1* knock-out profile is indicated at the lower right. Thus, simultaneously measuring induction of *PGU1* above 2 natural log ratios and repression of *DAK1* below one natural ratio specifically indicates Hog1p pathway inactivation.

Figure 75. The plot description is the same as for **Figure 74**. The subset of treatments that target mitochondrial function form a distinct group in the upper right quadrant (within rectangle). Thus, simultaneously measuring induction of *YGL198w* and *STE18* should specifically indicate perturbations of the mitochondria.

DETAILED DESCRIPTION OF THE INVENTION

Definitions and General Techniques

Unless otherwise defined, all technical and scientific terms used herein have the meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The practice of the present invention employs, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA, genetics and immunology. See, e.g., Maniatis et al., 1982; Sambrook et al., 1989; Ausubel et al., 1992; Glover, 1985; Anand, 1992; Guthrie and Fink, 1991 (which are incorporated herein by reference).

A "regulon" is a group of genes that are coordinately regulated in response to a number of different stimuli, e.g., treatment with chemical compounds or mutations. The member genes of a regulon comprise a functional unit by which a cell is able to adapt to a changing environment. The regulation of these genes that led to their categorization could be at the level of transcription, mRNA stability, splicing,

translation or protein stability. The mode of regulation of each member gene of a given regulon need not be the same.

Genes are categorized into separate regulons based upon changes in gene expression. In order to efficiently and accurately group genes into functional groups, it is necessary to observe each gene's expression change. Since many genes function in specialized roles, it is necessary to measure global gene expression under as diverse a variety of conditions as possible. Therefore, the database of expression profiles used in this invention was made from a diverse collection of chemicals and mutant strains of yeast. In general, the greater the number of diverse stimuli which cause the genes of a regulon to exhibit coordinate expression and the higher the correlation coefficient, the more confident one will be that the regulon is a robust indicator of the pathway or process of interest.

A "regulon indicator gene" (RIG) is a gene whose expression changes when a particular regulon or biochemical pathway or cellular process is activated or repressed. Although a RIG's expression may correlate with a particular biochemical pathway, the RIG does not necessarily have to be a part of the biochemical pathway for which it is an indicator. A RIG may comprise the entire gene, the 5' region of the gene including the promoter and/or enhancer and all or a part of the coding region, or a fragment, conservatively modified variant or homolog thereof which retains the indicator function of the RIG. A RIG may be coordinately expressed with a particular biological pathway, such that when the pathway is activated the RIG is more highly expressed and when the pathway is repressed the RIG's expression is repressed as well. However, the invention also encompasses RIGs in which there is an inverse correlation with a particular pathway. In this case, activation of a pathway would lead to a repression of RIG expression, while repression of a pathway would lead to activation of RIG expression. A RIG may be coordinately expressed with a particular biological pathway, such that when the pathway is activated the RIG is more highly expressed. However, the invention also encompasses RIGs in which there is an inverse correlation with a particular pathway. In this case, activation of a pathway would lead to a repression of RIG expression. Furthermore, the invention also encompasses RIGs

which are not necessarily part of the regulon, pathway or process for which they are indicators. In this case, expression of RIGs may be activated or repressed specifically in response to perturbations of a regulon, pathway or process even though the RIG itself may only be indirectly related or have no apparent relationship in function to the regulon, pathway or process.

In a preferred embodiment, a RIG is specific to a particular pathway, wherein its expression changes most significantly when a particular pathway is activated or repressed. Such a highly specific regulon indicator gene cannot always be found for a pathway of interest. In such cases, more than one RIG can be identified that, when their expression patterns are taken together, correlate with specificity to the pathway of interest. Thus, in another preferred embodiment, a plurality of RIGs is identified wherein the coordinated expression pattern of the plurality of RIGs is specific to a particular biological pathway. In this preferred embodiment, expression of each member of the plurality of RIGs may independently increase or decrease when the biological pathway of interest is activated or repressed.

In another preferred embodiment, a RIG is highly sensitive to changes in activation or repression of a pathway, such that even a small perturbation in regulation of a pathway results in a change in RIG expression. In a further preferred embodiment, a RIG has a large dynamic range, and is highly induced or repressed upon the corresponding perturbation of the pathway to which it is correlated.

In another preferred embodiment, a RIG does not contain sequences that are problematic for maintaining on plasmids when introduced into host cells. Such sequences that may be problematic include centromeric sequences or sites that are particularly susceptible to recombination.

A "target gene" or "regulon target gene" is a gene whose function is desirable to modulate. A target gene may consist of the entire gene, the 5' region comprising the promoter and/or enhancer and all or a part of the coding region, or a fragment, conservatively modified variant or homolog thereof which retains the function of the target gene. In general, a target gene encodes a protein which is a part of the biological (e.g., metabolic or biochemical) pathway or process whose

modulation would result in a desired outcome. In a preferred embodiment, a target gene is a control point in such a pathway. In one more preferred embodiment, a target gene is a control point that is relatively "upstream" in the metabolic pathway. "Upstream" means that the target gene is involved in one of the first steps of the metabolic pathway or process. In another more preferred embodiment, a target gene is a control point that is relatively "downstream" but specific to a biological pathway or a branch of that pathway or process. "Downstream" means that the target gene is involved in one of the later steps of the pathway or process.

A "target" or "target protein" is a protein whose expression or activity is to be modulated. A target may consist of the entire protein or a fragment, mutein, derivative or homolog thereof which retains the function of the target. In general, a target is a protein included within a biological pathway wherein it is desired to modulate the process which the protein is involved in. In a preferred embodiment, a target is a control point in such a biological pathway. In a more preferred embodiment, a target is a control point that is relatively "upstream" in the biological pathway. "Upstream" means that the target is involved in one of the first steps of the pathway. In another more preferred embodiment, a target is a control point that is relatively "downstream" but specific to a biological pathway or a branch of that pathway. "Downstream" means that the target is involved in one of the later steps of the pathway.

A "target-dependent reporter gene" is a gene whose expression is altered in a cell in which the target gene has been altered or inactivated compared to the cell which expresses the normal target gene. The expression of the target-dependent reporter gene may increase or decrease in a cell harboring an altered or inactivated target gene, depending upon the identity of the gene. If expression of the target-dependent reporter gene increases in the cell harboring the altered or inactivated target gene, then a potential inhibitor of the regulon target gene will increase expression of the target-dependent reporter gene, and if expression of the target-dependent reporter gene decreases in the cell, then a potential inhibitor of the regulon target gene will decrease expression of the target-dependent reporter gene.

By "pathway" is meant any biological, e.g., metabolic or biochemical, set of concerted reactions which occur in response to a particular signal or stimulus in a cell. The isoprenoid pathway is one example of such a pathway. Other pathways include, without limitation, amino acid and protein synthesis, lipid synthesis, protein and lipid glycosylation, protein modification, DNA synthesis and repair, RNA transcription, phospholipid synthesis, nucleotide synthesis, and energy generation and storage (e.g., glycolysis, citric acid cycle, oxidative phosphorylation, gluconeogenesis, pentose phosphate pathway, fatty acid metabolism, glycogen and disaccharide metabolism, amino acid degradation and the urea cycle), signal transduction and growth control.

By "process" is meant any biological reaction or set of reactions that occurs within a cell or organism that occurs in response to a stimulus or signal, or that occurs during growth, homeostasis, development, differentiation or death of the cell or organism.

An "isolated" protein or polypeptide is one that has been separated from naturally associated components that accompany it in its native state. Thus, a polypeptide that is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be "isolated" from its naturally associated components. A protein may also be rendered substantially free of naturally associated components by isolation, using protein purification techniques well known in the art.

A monomeric protein is "substantially pure," "substantially homogeneous" or "substantially purified" when at least about 60 to 75% of a sample exhibits a single polypeptide sequence. A substantially pure protein will typically comprise about 60 to 90% W/W of a protein sample, more usually about 95%, and preferably will be over 99% pure. Protein purity or homogeneity may be indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualizing a single polypeptide band upon staining the gel with a stain well known in the art. For certain purposes, higher resolution may be provided by using HPLC or other means well known in the art for purification.

A *S. cerevisiae* protein has "homology" or is "homologous" to a protein from another organism if the encoded amino acid sequence of the yeast protein has a similar sequence to the encoded amino acid sequence of a protein of a different organism. Alternatively, a *S. cerevisiae* protein may have homology or be homologous to another *S. cerevisiae* protein if the two proteins have similar amino acid sequences. Although two proteins are said to be "homologous," this does not imply that there is necessarily an evolutionary relationship between the proteins. Instead, the term "homologous" is defined to mean that the two proteins have similar amino acid sequences. In addition, although in many cases proteins with similar amino acid sequences will have similar functions, the term "homologous" does not imply that the proteins must be functionally similar to each other.

When "homologous" is used in reference to proteins or peptides, it is recognized that residue positions that are not identical often differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of homology may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art (see, e.g., Pearson et al.,1994, and [Henikoff et al., 1992, herein incorporated by reference).

The following six groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Serine (S), Threonine (T);
2) Aspartic Acid (D), Glutamic Acid (E);
3) Asparagine (N), Glutamine (Q);
4) Arginine (R), Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V), and

6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

Sequence homology for polypeptides, which is also referred to as sequence identity, is typically measured using sequence analysis software. See, e.g., the Sequence Analysis Software Package of the Genetics Computer Group (GCG), University of Wisconsin Biotechnology Center, 910 University Avenue, Madison, Wisconsin 53705. Protein analysis software matches similar sequences using measure of homology assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG contains programs such as "Gap" and "Bestfit" which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof.

A preferred algorithm when comparing a *S. cerevisiae* sequence to a database containing a large number of sequences from different organisms is the computer program BLAST, especially blastp or tblastn (Altschul et al., 1997, herein incorporated by reference). Preferred parameters for blastp are:

Expectation value:	10 (default)
Filter:	seg (default)
Cost to open a gap:	11 (default)
Cost to extend a gap:	1 (default
Max. alignments:	100 (default)
Word size:	11 (default)
No. of descriptions:	100 (default)
Substitution Matrix:	BLOSUM62

The length of polypeptide sequences compared for homology will generally be at least about 16 amino acid residues, usually at least about 20 residues, more usually at least about 24 residues, typically at least about 28 residues, and preferably more than about 35 residues. When searching a database containing sequences from a large number of different organisms using a *S. cerevisiae* query sequence, it is preferable to compare amino acid sequences. Comparison of amino acid sequences is preferred to comparing nucleotide sequences because *S. cerevisiae* has significantly different codon usage compared to mammalian or plant codon usage.

Database searching using amino acid sequences can be measured by

algorithms other than blastp known in the art. For instance, polypeptide sequences can be compared using Fasta, a program in GCG Version 6.1. Fasta provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, 1990, herein incorporated by reference). For example, percent sequence identity between amino acid sequences can be determined using Fasta with its default parameters (a word size of 2 and the PAM250 scoring matrix), as provided in GCG Version 6.1, herein incorporated by reference.

The invention envisions two general types of polypeptide "homologs." Type 1 homologs are strong homologs. A comparison of two polypeptides that are Type 1 homologs would result in a blastp score of less than $1x10^{-40}$, using the blastp algorithm and the parameters listed above. The lower the blastp score, that is, the closer it is to zero, the better the match between the polypeptide sequences. For instance, yeast lanosterol demethylase, which is a common target of antifungal agents, as discussed above, has a Type 1 homolog in humans. The probability score (e.g., blastp score) is dependent upon the size of the database. Comparison of yeast and human lanosterol demethylases produces a blastp score of $1x10^{-86}$.

Type 2 homologs are weaker homologs. A comparison of two polypeptides that are Type 2 homologs would result in a blastp score of between $1x10^{-40}$ and $1x10^{-10}$, using the Blast algorithm and the parameters listed above. One having ordinary skill in the art will recognize that other algorithms can be used to determine weak or strong homology.

The terms "no substantial homology" or "no human (or mammalian, vertebrate, amphibian, fish, insect or plant) homolog" refers to a yeast polypeptide sequence which exhibits no substantial sequence identity with a polypeptide sequence from human, non-human mammals, other vertebrates, insects or plants. A comparison of two polypeptides which have no substantial homology to one another would result in a blastp score of greater than $1x10^{-10}$, using the Blast algorithm and the parameters listed above. One having ordinary skill in the art will recognize that other algorithms can be used to determine whether two polypeptides demonstrate no substantial homology to each other.

A polypeptide "fragment," "portion" or "segment" refers to a stretch of amino acid residues of at least about five to seven contiguous amino acids, often at least about seven to nine contiguous amino acids, typically at least about nine to 13 contiguous amino acids and, most preferably, at least about 20 to 30 or more contiguous amino acids.

A polypeptide "mutein" refers to a polypeptide whose sequence contains substitutions, insertions or deletions of one or more amino acids compared to the amino acid sequence of the native or wild type protein. A mutein has at least 50% sequence homology to the wild type protein, preferred is 60% sequence homology, more preferred is 70% sequence homology. Most preferred are muteins having 80%, 90% or 95% sequence homology to the wild type protein, in which sequence homology is measured by any common sequence analysis algorithm, such as Gap or Bestfit.

A "derivative" refers to polypeptides or fragments thereof that are substantially homologous in primary structural sequence but which include, e.g., *in vivo* or *in vitro* chemical and biochemical modifications or which incorporate unusual amino acids. Such modifications include, for example, acetylation, carboxylation, phosphorylation, glycosylation, ubiquitination, labeling, e.g., with radionuclides, and various enzymatic modifications, as will be readily appreciated by those well skilled in the art. A variety of methods for labeling polypeptides and of substituents or labels useful for such purposes are well known in the art, and include radioactive isotopes such as ^{125}I, ^{32}P, ^{35}S, and ^{3}H, ligands which bind to labeled antiligands (e.g., antibodies), fluorophores, chemiluminescent agents, enzymes, and antiligands which can serve as specific binding pair members for a labeled ligand. The choice of label depends on the sensitivity required, ease of conjugation with the primer, stability requirements, and available instrumentation. Methods for labeling polypeptides are well known in the art. See Ausubel et al., 1992, hereby incorporated by reference.

The term "fusion protein" refers to polypeptides comprising polypeptides or fragments coupled to heterologous amino acid sequences. Fusion proteins are useful because they can be constructed to contain two or more desired

functional elements from two or more different proteins. Fusion proteins can be produced recombinantly by constructing a nucleic acid sequence which encodes the polypeptide or a fragment thereof in frame with a nucleic acid sequence encoding a different protein or peptide and then expressing the fusion protein. Alternatively, a fusion protein can be produced chemically by crosslinking the polypeptide or a fragment thereof to another protein.

An "isolated" or "substantially pure" nucleic acid or polynucleotide (e.g., an RNA, DNA or a mixed polymer) is one which is substantially separated from other cellular components that naturally accompany the native polynucleotide in its natural host cell, e.g., ribosomes, polymerases, or genomic sequences with which it is naturally associated. The term embraces a nucleic acid or polynucleotide that has been removed from its naturally occurring environment. The term "isolated" or "substantially pure" also can be used in reference to recombinant or cloned DNA isolates, chemically synthesized polynucleotide analogs, or polynucleotide analogs that are biologically synthesized by heterologous systems.

The term "percent sequence identity" or "identical" in the context of nucleic acid sequences refers to the residues in the two sequences which are the same when aligned for maximum correspondence. The length of sequence identity comparison may be over a stretch of at least about nine nucleotides, usually at least about 20 nucleotides, more usually at least about 24 nucleotides, typically at least about 28 nucleotides, more typically at least about 32 nucleotides, and preferably at least about 36 or more nucleotides. There are a number of different algorithms known in the art which can be used to measure nucleotide sequence identity. For instance, polynucleotide sequences can be compared using Fasta, a program in GCG Version 6.1. Fasta provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, 1990, herein incorporated by reference). For instance, percent sequence identity between nucleic acid sequences can be determined using Fasta with its default parameters (a word size of 6 and the NOPAMfactor for the scoring matrix) as provided in GCG Version 6.1, herein incorporated by reference.

The term "substantial homology" or "substantial similarity," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 60% of the nucleotide bases, usually at least about 70%, more usually at least about 80%, preferably at least about 90%, and more preferably at least about 95-98% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as Fasta, as discussed above.

Alternatively, substantial homology or similarity exists when a nucleic acid or fragment thereof hybridizes to another nucleic acid, to a strand of another nucleic acid, or to the complementary strand thereof, under selective hybridization conditions. Typically, selective hybridization will occur when there is at least about 55% sequence identity -- preferably at least about 65%, more preferably at least about 75%, and most preferably at least about 90% -- over a stretch of at least about 14 nucleotides. See, e.g., Kanehisa, 1984, herein incorporated by reference.

Nucleic acid hybridization will be affected by such conditions as salt concentration, temperature, solvents, the base composition of the hybridizing species, length of the complementary regions, and the number of nucleotide base mismatches between the hybridizing nucleic acids, as will be readily appreciated by those skilled in the art. "Stringent hybridization conditions" and "stringent wash conditions" in the context of nucleic acid hybridization experiments depend upon a number of different physical parameters. The most important parameters include temperature of hybridization, base composition of the nucleic acids, salt concentration and length of the nucleic acid. One having ordinary skill in the art knows how to vary these parameters to achieve a particular stringency of hybridization. In general, "stringent hybridization" is performed at about 25°C below the thermal melting point (T_m) for the specific DNA hybrid under a particular set of conditions. "Stringent washing" is performed at temperatures about 5°C lower than the T_m for the specific DNA hybrid under a particular set of conditions. The T_m is the temperature at which 50% of the target sequence hybridizes to a perfectly matched probe. See Sambrook et al., page

9.51, hereby incorporated by reference.

The T_m for a particular DNA-DNA hybrid can be estimated by the formula:

$T_m = 81.5°C + 16.6 (\log_{10}[Na^+]) + 0.41$ (fraction G + C) - 0.63 (% formamide) - (600/l) where l is the length of the hybrid in base pairs.

The T_m for a particular RNA-RNA hybrid can be estimated by the formula:

$T_m = 79.8°C + 18.5 (\log_{10}[Na^+]) + 0.58$ (fraction G + C) + 11.8 (fraction G + C)^2 - 0.35 (% formamide) - (820/l).

The T_m for a particular RNA-DNA hybrid can be estimated by the formula:

$T_m = 79.8°C + 18.5(\log_{10}[Na^+]) + 0.58$ (fraction G + C) + 11.8 (fraction G + C)^2 - 0.50 (% formamide) - (820/l).

In general, the T_m decreases by 1-1.5°C for each 1% of mismatch between two nucleic acid sequences. Thus, one having ordinary skill in the art can alter hybridization and/or washing conditions to obtain sequences that have higher or lower degrees of sequence identity to the target nucleic acid. For instance, to obtain hybridizing nucleic acids that contain up to 10% mismatch from the target nucleic acid sequence, 10-15°C would be subtracted from the calculated T_m of a perfectly matched hybrid, and then the hybridization and washing temperatures adjusted accordingly. Probe sequences may also hybridize specifically to duplex DNA under certain conditions to form triplex or other higher order DNA complexes. The preparation of such probes and suitable hybridization conditions are well known in the art.

An example of stringent hybridization conditions for hybridization of complementary nucleic acid sequences having more than 100 complementary residues on a filter in a Southern or Northern blot or for screening a library is 50% formamide/6X SSC at 42°C for at least ten hours. Another example of stringent hybridization conditions is 6X SSC at 68°C for at least ten hours. An example of low stringency hybridization conditions for hybridization of complementary nucleic acid sequences having more than 100 complementary residues on a filter in a Southern or

northern blot or for screening a library is 6X SSC at 42°C for at least ten hours. Hybridization conditions to identify nucleic acid sequences that are similar but not identical can be identified by experimentally changing the hybridization temperature from 68°C to 42°C while keeping the salt concentration constant (6X SSC), or keeping the hybridization temperature and salt concentration constant (e.g. 42°C and 6X SSC) and varying the formamide concentration from 50% to 0%. Hybridization buffers may also include blocking agents to lower background. These agents are well-known in the art. See Sambrook et al., pages 8.46 and 9.46-9.58, herein incorporated by reference.

Wash conditions also can be altered to change stringency conditions. An example of stringent wash conditions is a 0.2x SSC wash at 65°C for 15 minutes (see Sambrook et al., for SSC buffer). Often the high stringency wash is preceded by a low stringency wash to remove excess probe. An exemplary medium stringency wash for duplex DNA of more than 100 base pairs is 1x SSC at 45°C for 15 minutes. An exemplary low stringency wash for such a duplex is 4x SSC at 40°C for 15 minutes. In general, signal-to-noise ratio of 2x or higher than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization.

As defined herein, nucleic acids that do not hybridize to each other under stringent conditions are still substantially homologous to one another if they encode polypeptides that are substantially identical to each other. This occurs, for example, when a nucleic acid is created synthetically or recombinantly using a high codon degeneracy as permitted by the redundancy of the genetic code.

The polynucleotides of this invention may include both sense and antisense strands of RNA, cDNA, genomic DNA, and synthetic forms and mixed polymers of the above. They may be modified chemically or biochemically or may contain non-natural or derivatized nucleotide bases, as will be readily appreciated by those of skill in the art. Such modifications include, for example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as uncharged linkages (e.g., methyl phosphonates,

phosphotriesters, phosphoramidates, carbamates, etc.), charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), pendent moieties (e.g., polypeptides), intercalators (e.g., acridine, psoralen, etc.), chelators, alkylators, and modified linkages (e.g., alpha anomeric nucleic acids, etc.) Also included are synthetic molecules that mimic polynucleotides in their ability to bind to a designated sequence via hydrogen bonding and other chemical interactions. Such molecules are known in the art and include, for example, those in which peptide linkages substitute for phosphate linkages in the backbone of the molecule.

"Conservatively modified variations" or "conservatively modified variants" of a particular nucleic acid sequence refers to nucleic acids that encode identical or essentially identical amino acid sequences or DNA sequences where no amino acid sequence is encoded. Due to the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given polypeptide sequence. When a nucleic acid sequence is changed at one or more positions with no corresponding change in the amino acid sequence which it encodes, that mutation is called a "silent mutation." Thus, one species of a conservatively modified variation according to this invention is a silent mutation. Accordingly, every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent mutation or variation.

Furthermore, one of skill in the art will recognize that individual substitutions, deletions, additions and the like, which alter, add or delete a single amino acid or a small percentage of amino acids (less than 5%, more typically less than 1%) in an encoded sequence are "conservatively modified variations" or "conservatively modified variants" where the alterations result in the substitution of one amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art.

The term "antibody" refers to a polypeptide encoded by an immunoglobulin gene, genes, or fragments thereof. The immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant regions, as well as a myriad of immunoglobulin variable regions. Light chains are classified as either kappa

or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes IgG, IgM, IgA, IgD and IgE, respectively.

Antibodies exist for example, as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. For example, trypsin digests an antibody below the disulfide linkages in the hinge region to produce $F(ab)'_2$, a dimer of Fab which itself is a light chain joined to a V_H-C_H1 by a disulfide bond. The $F(ab)'_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge region thereby converting the $F(ab)'_2$ dimer to a Fab' monomer. The Fab' monomer is essentially an Fab with part of the hinge region. See Paul (1993) (incorporated herein by reference), for a detailed description of epitopes, antibodies and antibody fragments. One of skill in the art recognizes that such Fab' fragments may be synthesized *de novo* either chemically or using recombinant DNA technology. Thus, as used herein, the term antibody includes antibody fragments produced by the modification of whole antibodies or those synthesized de novo. The term antibody also includes single-chain antibodies, which generally consist of the variable domain of a heavy chain linked to the variable domain of a light chain. The production of single-chain antibodies is well known in the art (see, e.g., U.S. Pat. No. 5,359,046). The antibodies of the present invention are optionally derived from libraries of recombinant antibodies in phage or similar vectors (see, e.g., Huse et al. (1989); Ward et al. (1989); Vaughan et al. (1996) which are incorporated herein by reference).

As used herein, "epitope" refers to an antigenic determinant of a polypeptide, i.e., a region of a polypeptide that provokes an immunological response in a host. This region need not comprise consecutive amino acids. The term epitope is also known in the art as "antigenic determinant." An epitope may comprise as few as three amino acids in a spatial conformation which is unique to the immune system of the host. Generally, an epitope consists of at least five such amino acids, and more usually consists of at least 8-10 such amino acids. Methods for determining the spatial conformation of such amino acids are known in the art.

Methods for Analyzing ORF Gene Expression

The cell's ability to monitor its own biochemical ecology may be considered as a fully integrated multi-dimensional set of specific biochemical assays. The data from each individual assay manifests itself either directly or indirectly in the change in expression of a single gene or small set of genes. The individual components of the assaying capabilities of the cell may be extracted by measuring the changes in global gene expression in response to a controlled experimental challenge.

The measurement of global gene expression may be done by a number of different methods. One technique is that of hybridization to nucleic acid arrays on solid surfaces, such as "gene chips" (Fodor et al., 1991). Another method uses a reporter construct in the GRM or an equivalent matrix comprising living cells, preferably eukaryotic cells, and more preferably yeast, insect, plant, avian, fish or mammalian cultured cells. Other methods include SAGE.

DNA Chip Technology

One method for determining comprehensive gene expression profiles is DNA gene chip technology (see, e.g., Fodor et al., 1991). A DNA gene chip can be made comprising a large number of immobilized single-stranded nucleic acids, each of which hybridizes specifically to a gene or its mRNA, representing a particular genome or a significant subset thereof. Messenger RNA molecules extracted from a cell or cDNA molecules converted from such mRNA molecules can be labeled. The labeling can be accomplished, for example, radioisotopically or fluorescently by methods well known in the art. These mRNA or cDNA molecules are rendered single-stranded and then allowed to hybridize to the immobilized single-stranded nucleic acids on the gene chip. A computer equipped with a scanner then determines the extent of hybridization, thereby quantitating the amount of mRNA produced for any given gene or genetic sequence.

Profiles of gene expression generated under different conditions or in response to different stimuli such as treatment with chemical compounds are produced by treating cells with a compound, isolating the mRNA the cells, optionally producing

cDNA and then hybridizing the single-stranded nucleic acids on the gene chip as discussed above. Preferably, software is used to correlate the expression of each gene on the hybridization chip relative to other genes under different conditions or in response to different treatments (see below).

Promoter elements from genes of interest that respond to an input signal can then be isolated and operatively linked to a reporter gene described above by recombinant DNA techniques well known in the art for further characterization.

Genome Reporter Matrix™ Technology

An alternative method to DNA gene chip technology is the use of a Genome Reporter Matrix™ (GRM), or an equivalent thereof. The description below of the generation of gene expression profiles utilizing the Genome Reporter Matrix™ has been described essentially in United States Patents 5,569,888 and 5,777,888, both of which are incorporated herein by reference.

The promoter (and optionally, 5' upstream regulatory elements and/or 5' upstream untranslated sequences) of an ORF or a gene from a cellular genome (preferably a eukaryotic genome) is fused to a reporter gene creating a transcriptional and/or translational fusion of the promoter to the reporter gene. In a preferred embodiment, the genome is that of *S. cerevisiae*. The promoter and optional additional sequences comprise all the regulatory elements necessary for transcriptional (and optionally translational) control of an attached coding sequence. The reporter gene can be any gene that, when expressed in a suitable host, encodes a product that can be detected by a quantitative assay. Any suitable assay may be used, including but not limited to enzymatic, colorimetric, fluorescence or other spectrographic assays, fluorescent activated cell sorting assay and immunological assays. Examples of suitable reporter genes include, inter alia, green fluorescent protein (GFP), β-lactamase, lacZ, invertase, membrane bound proteins (e.g., CD2, CD4, CD8, the influenza hemagglutinin protein, and others well known in the art) to which high affinity antibodies directed to them exist or can be made routinely, fusion protein comprising membrane bound protein appropriately fused to an antigen tag domain

(e.g., hemagglutinin or Myc and others well known in the art). In a preferred embodiment, the reporter protein is GFP from the jellyfish *Aequorea victoria.* GFP is a naturally fluorescing protein that does not require the addition of any exogenous substrates for activity. The ability to measure GFP fluorescence in intact living cells makes it an ideal reporter protein for the GRM or an equivalent matrix comprising living cells.

In a preferred embodiment, reporter constructs comprise the 5' region of the ORF comprising the promoter of the ORF and other expression regulatory sequences, and generally the first four codons of the ORF fused in-frame to the green fluorescent protein. In a more preferred embodiment, approximately 1200 base-pairs of 5' regulatory sequence are included in each fusion. Only 228 yeast ORFs (3.5%) possess introns. Of these 228 intron-containing ORFs, all but four contain only one intron. In these ORFs, fusions are created two to four codons past (3' to) the splice junction. Therefore, these fusions must undergo splicing in order to create a functional reporter fusion.

Each reporter is assembled in an episomal yeast shuttle vector (either CEN or 2μ plasmid) or on a yeast integrating vector for subsequent insertion into the chromosomal DNA. In a preferred embodiment, the gene reporter constructs are built using a yeast multicopy vector. A multicopy vector is chosen to facilitate easy transfer of the reporter constructs to many different yeast strain backgrounds. In addition, the vector replicates at an average of 10 to 20 copies per cell, providing added sensitivity for detecting genes that are expressed at a low level. In principle, introducing additional copies of a gene's regulatory region could, through titration of regulatory proteins, disrupt a response of interest. However, in practice this appears not to occur, and efforts to successfully exploit such titration effects have required much higher copy number vectors and have been largely unsuccessful. In another preferred embodiment, the reporter constructs are maintained on episomal plasmids in yeast.

In one embodiment, a plurality (all or a significant subset) of the resulting approximately 6,000 reporter constructs is transformed into a strain of yeast. The resulting strains constitute one embodiment of the Genome Reporter Matrix™.

See Example 1.

Profiles are produced by arraying wild type or mutant cells carrying the reporter fusion genes in growth media containing different drugs and chemical compounds and measuring changes in expression of the reporter gene by the appropriate assay (see below). In a preferred embodiment, where the reporter gene is GFP, measurement of changes in expression are done by measuring the amount of green light produced by the cells over time with an automated fluorescence scanner. Alternatively, the drugs or chemical compounds may be added to the yeast cells after they have been arrayed onto growth media and then measuring changes in reporter gene expression by the appropriate assay.

Over 93% of the reporters are detectable over background on rich medium. The reproducibility of individual reporters is high, with expression generally varying by less than 10%. In contrast, hybridization experiments have proven unreliable for effects of less than a factor of two. **Figure 64** depicts expression data of the GRM from two independent experiments plotted against each other.

In a preferred embodiment, the GRM is used to obtain gene expression information from a genome. The GRM is preferred to hybridization-based methods of profiling for several reasons. First, because the promoter-reporter fusions include the first four amino acids of the native gene product, the response profiles are composites of both transcriptional and translational effects. The importance of being able to monitor both levels of response is underscored by the experience with bacterial antibiotics. Those antibiotics that work at the translational level have a greater therapeutic performance than those affecting transcription. Because hybridization-based methods can reveal only effects on transcription, profiling with the GRM provides a more complete view of the full spectrum of biological effects induced by exposure to drugs or compounds.

Second, the GRM permits profiling of gene expression changes in living cells, which permits one to easily measure the kinetics of changes in gene response profiles in the same population of cells following exposure to different drugs and

chemical agents. Thus, by collecting multiple data sets over time, one can identify the genes that make up primary and secondary responses.

Third, hybridization-based methods require relatively sophisticated molecular procedures to produce labeled cDNA, followed by a 14 hour hybridization of labeled cDNA probes to target DNA arrays on slides or chips. The GRM requires only that being able to produce arrays of colonies and measure emitted light. These procedures are easier to scale up in an industrial setting than are sophisticated molecular biology methods, rendering data that is more straightforward to produce and more reproducible in nature.

Gene Expression Profiles

Using the reporter construct, gene chip technology or another method for obtaining genome-wide gene expression, the gene expression profile of yeast genes can be obtained. In a preferred embodiment, either the GRM or gene chip technology is used. In a more preferred embodiment, the GRM is treated with a number of pharmaceutical compounds and the resulting expression of the reporter constructs is analyzed. Generally, for each pharmaceutical compound, the expression of the reporter constructs are analyzed in the presence of the vehicle for the pharmaceutical compound alone and is compared to the expression of the reporter constructs in the presence of the pharmaceutical compound. Changes in expression of the reporter constructs in the absence and presence of the pharmaceutical compound is obtained either by subtracting the baseline level of expression from the level after treatment or dividing the baseline level of expression from the level after treatment. By looking at a large number of reporter constructs, one can assign yeast ORFs to functional groups based upon their expression patterns in response to various pharmaceutical compounds. These functional groups may provide valuable information as to the function of the yeast proteins as well as their human, non-human mammalian, avian, fish, insect and plant counterparts.

Preferably, software is used to correlate the expression of each gene in the GRM or on the DNA chip relative to other genes under different conditions and in

response to different pharmaceutical compounds. In one preferred embodiment, the software is capable of producing a correlation coefficient for each gene's expression relative to every other gene across all expression profiles in a database. Such analysis reveals groups of genes that exhibit coordinate regulation (regulons). See, e.g., U.S. Serial No. 09/076,668, now pending; Eisen et al. (1998); and Tamayo et al. (1999).

In a preferred embodiment, a gene of unknown function may be placed into a functional genetic group by the following steps:

a) generating a gene expression profile for Gene X, a gene of unknown function;

b) comparing the gene expression profile of Gene X with expression profiles of a plurality of other genes in a database of compiled gene expression profiles to generate expression correlation coefficients;

c) identifying based on their expression correlation coefficients a set of genes comprising Gene X that are coordinately expressed;

d) determining if the genes whose expression is most highly correlated with that of Gene X belong to a gene regulon involved in a known biological pathway, or a common set of biological reactions or functions; and

e) optionally testing the effect on Gene X expression of at least one altered condition or treatment known to affect the function to which Gene X hs been ascribed.

If Gene X expression is coordinate with expression of the regulon, then Gene X is placed in the regulon.

Methods to Identify Potential RIGs

A GRM (or an equivalent) is chemically treated with a large number of compounds. Regulons are identified as groups of genes that are coordinately regulated in response to genetic mutations, treatment with compounds or different environmental conditions. In a preferred embodiment, regulons are identified using correlation

coefficients assembled by software that does clustering analysis, such as that described in U.S. Serial No. 09/076,668, now pending; Eisen et al. (1998); and Tamayo et al. (1999). In a preferred embodiment, genes that constitute a regulon have a correlation coefficient of greater than 0.5. In a more preferred embodiment, genes that constitute a regulon have a correlation coefficient of at least 0.6 or 0.7. In a further preferred embodiment, genes that constitute a regulon have a correlation coefficient of at least 0.8 or 0.9. The correlation coefficient may be measured by any method of obtaining correlation coefficients, including, without limitation, the method described in United States Patent Application Serial No. 09/076,668, now pending or in Eisen et al. (1998).

Once a group of genes has been grouped into a regulon, one can identify potential regulon indicator genes (RIGs), which may or may not be a member of the regulon, pathway or process with the regulon, pathway, or process for which they are an indicator. RIGs may be either characterized or uncharacterized genes provided they have certain characteristics. Preferred characteristic include one or more of the following: 1) its expression profile is sensitive to one or more stimuli; 2) its expression profile exhibits a large dynamic range in response to one or more stimuli; 3) its expression profile exhibits a rapid kinetic response to one or more stimuli; 4) its expression profile is specific to a known biological pathway or a common set of biological reactions or functions; 5) the regulon indicator gene does not contain sequences that are problematic for maintaining on plasmids when introduced into host cells. Most preferably, their expression is relatively specific for a particular biochemical pathway or cellular condition, highly sensitive to small changes in activation of a biochemical pathway or cellular condition and exhibit a wide dynamic range of expression so that the RIG is easier to assay.

A "large dynamic range" is one in which the response in gene expression in response to a stimulus is at least four-fold over basal levels of expression in the absence of the stimulus. A response may be either an increase or a decrease in gene expression. In a preferred embodiment, the response is at least ten-fold over basal levels. In a more preferred embodiment, the response is at least twenty-fold over

basal levels. In an even more preferred embodiment, the response is at least 100-fold over basal levels.

A "rapid kinetic response" is one in which the response occurs in the same time period as the doubling time of the organism after stimulation with the stimulus. In a preferred embodiment, the response occurs less than 10 minutes. In a more preferred embodiment, the response occurs in less than one minute.

A "stimulus" or "stimuli" is a chemical compound, a genetic mutation, or a change in the environment of the cell, including, without limitation, a change in pH, temperature, osmotic pressure, salinity, light, gas concentration or partial pressure (e.g. O_2, CO_2, CO or NO).

In order to determine whether a potential RIG is specific for a particular biochemical pathway or cellular condition, expression of the potential RIG is examined under all conditions in the expression database. A desirable RIG is one whose expression is selectively induced or repressed by chemicals or mutations that are known to affect the process in question. Likewise, a desirable RIG's expression is not influenced by chemicals or mutations that are known not to affect the process in question. This analysis provides information regarding whether the RIG participates in additional cellular processes or biochemical pathways. When a potential RIG is not a member of a target regulon, pathway or process, specificity is measured by analyzing expression under all conditions under which the potential RIG is activated or repressed to determine if similar conditions elicit similar responses.

Most preferably, a single RIG may be identified to be highly specific to a particular pathway, i.e., wherein its expression changes only when a particular pathway is activated or repressed, but not when other pathways are likewise regulated. Such a highly specific regulon indicator gene cannot always be found for a pathway of interest. In such cases, however, more than one RIG may be identified whose coordinate expression patterns correlate with high specificity to a pathway of interest. Preferably, the coordinate expression of two RIGs provides such specificity. However, the present invention is not limited by the number of RIGs identified and used simultaneously as regulated pathway indicators. Expression of each member of a

plurality of RIGs may independently increase or decrease when the biological pathway of interest is activated or repressed.

In order to determine whether a potential RIG is highly indicative of activation of a particular pathway, the gene will be activated or repressed to an expression level at least 2-fold higher or lower (if the gene is repressed) than when the pathway is not activated. In a preferred embodiment, the gene is activated or repressed to an expression level at least 10-fold higher or lower than the unactivated pathway. In a more preferred embodiment, the gene is activated or repressed to an expression level at least 20-fold higher or lower than the unactivated pathway. The expression level may be represented as a natural log ratio of treated/untreated expression values. See Figure 37, for example. In a preferred embodiment, the natural log ratio of a RIG is greater than 1, more preferably greater than 2.5, and even more preferably greater than 4.0 when the pathway or process is activated.

In order to determine the dynamic range of a potential RIG, the expression of the RIG is assessed by examining its expression in response to all the treatments and mutations in the database. In a preferred embodiment, there is a high level of change in RIG expression for small changes in activation of the pathway.

In one embodiment of the invention, expression of a regulon indicator gene correlates with the expression of at least one known gene in a group of coordinately expressed genes or provide a measure of the function of a biological process of interest. The RIG is identified by a method comprising the steps of:

a) comparing gene expression profiles of a plurality of genes in the database to generate expression correlation coefficients;

b) identifying based on their relative expression correlation coefficients a set of genes that are coordinately expressed;

c) selecting a set of genes from b) which comprises one or more genes known to function in a particular biological pathway, or a common set of biological reactions or functions;

d) selecting a member of the set of c) having one or more of the following characteristics:

1) its expression profile is sensitive to one or more stimuli;
2) its expression profile exhibits a large dynamic range in response to one or more stimuli;
3) its expression profile exhibits a rapid kinetic response to one or more stimuli;
4) its expression profile is specific to a known biological pathway or a common set of biological reactions or functions;
5) the regulon indicator gene does not contain sequences that are problematic for maintaining on plasmids when introduced into host cells.

The RIG may also be co-regulated with one or more genes in the group of coordinately expressed genes of c) above. In addition, the RIG may control the expression of at least one other gene in the group of coordinately expressed genes of c) above. The RIG may be a gene of previously unknown function.

In another embodiment, the invention provides a method for identifying a regulon indicator gene in a database of compiled gene expression profiles, wherein expression of the regulon indicator gene provides a measure of the function of a biological pathway or process of interest. The method comprises the steps of:

a) examining exemplary expression profiles in response to one or more chemical or genetic treatments which target the pathway or process of interest to generate reporter sensitivity data;

b) selecting a set of genes from a) which comprises one or more genes most significantly affected in response to the treatment or treatments; and

c) selecting at least one gene from b) whose expression profile is maximized for its specificity and sensitivity to the treatment or class of treatments in a) compared to its sensitivity to all other treatments in the database.

The regulon indicator gene may be co-regulated with one or more genes in the set of genes of a) or the regulon indicator gene, upon expression, controls the expression of at least one other gene in the in the set of genes of a).

Methods to Identify Potential Target Genes and Targets

A regulon is identified as described above under "Methods to Identify Potential RIGs." In a preferred embodiment, a regulon will contain both characterized and uncharacterized genes. In many cases, the characterized genes will have a common function or will be part of the same biochemical pathway. For instance, a regulon of the isoprenoid pathway will contain characterized genes involved in sterol biosynthesis. Uncharacterized genes will then be analyzed in terms of whether they are likely to be part of the same biochemical pathway as the characterized genes. The sequence of uncharacterized genes will be compared to the sequence of genes of known function to determine if the uncharacterized genes or their gene products have any motifs common to characterized genes.

For instance, uncharacterized genes will be examined for domains indicating enzymatic functions, including, without limitation, kinase, protease and phosphorylase activities. Similarly, uncharacterized genes will be examined for domains indicating that they might be transcription factors, including, without limitation, zinc finger, PHD, steroid-binding and helix-loop-helix regions. Other domains of interest include lipid-binding and ATP-binding domains. Uncharacterized genes will also be examined for sequence similarities to secreted factors and receptors. In a preferred embodiment, target genes and their encoded target proteins are previously uncharacterized, highly correlated with a particular regulon containing genes for a specific pathway or process, and that appear to be an enzyme, secreted factor, receptor or transcription factor.

In a preferred embodiment, a novel regulon target gene may be selected from a database of compiled gene expression profiles. The target gene is selected comprising the steps of:

a) comparing gene expression profiles of a plurality of genes in the database to generate expression correlation coefficients;

b) identifying based on their expression correlation coefficients a set of genes that are coordinately expressed;

c) selecting from b) a set of genes comprising one or more genes of unknown function and one or more genes known to function in a particular biological pathway, or a common set of biological reactions or functions of interest;

d) selecting from the set of c) at least one gene of unknown function, Gene X, as a novel regulon target gene; wherein Gene X is a gene whose expression profile closely correlates to the expression profiles of the one or more genes of the set of c) known to function in the particular biological pathway, or common set of biological reactions or functions of interest.

The method may further comprise the step of generating individual correlation coefficients between the gene expression profile of Gene X and a plurality of genes in the database to assess the selectivity of Gene X as a novel regulon target gene. The method may further comprise the step of determining whether the protein encoded by Gene X exhibits substantial homology to a human, non-human mammal, avian, amphibian, fish, insect or plant protein, including, without limitation, the step of hybridizing Gene X to genomic DNA from human, non-human mammal, avian, amphibian, fish, insect or plant cells or tissue under low stringency conditions, comparing the DNA sequence of Gene X to the DNA sequences from other organisms, or obtaining an amino acid sequence encoded by Gene X and comparing it to amino acid sequences from other organisms. The DNA or amino acid sequences from other organisms may be contained within a database and the DNA or amino acid sequence encoded by Gene X may compared to the DNA or amino acid sequences from other organisms using a computer algorithm such as blastp, tblastn or another algorithm that utilizes string alignments. The method for identifying a target may further comprise the steps of:

a) disrupting the function of Gene X or its homolog in a yeast cell; and

b) identifying whether the function of Gene X is essential for yeast germination, vegetative growth, pseudohyphal or hyphal growth.

In another embodiment of the invention, genes that are regulated by regulon target genes of yeast or its mammalian homolog may be identified. The method comprises the steps of

a) overexpressing the target gene in host cells of a matrix comprising a plurality of units of cells, the cells in each unit containing a reporter gene operably linked to an expression control sequence derived from a gene of a selected organism; and

b) identifying genes that are either induced or repressed by overexpression of the target gene.

In a preferred embodiment, the target gene is selected from the group consisting of *YMR134w*, *YER034w*, *YJL105w*, *YKL077w*, *YGR046w*, *YJR041c*, *YER044c* and *YLR100w* and their mammalian homologs.

Methods for Constructing Mutant Yeast Strains

Once a potential target has been identified, one may disrupt the gene to determine the effect of inhibiting the gene's activity has on the phenotype of the yeast cell. There are a number of methods well known in the art by which a person can disrupt a particular gene in yeast. One of skill in the art can disrupt an entire gene and create a null allele, in which no portion of the gene is expressed. One may also produce and express an allele comprising a portion of the gene which is not sufficient for gene function. This may be done by inserting a nonsense codon into the sequence of the gene such that translation of the mutant mRNA transcript ends prematurely. One may also produce and express alleles containing point mutations, individually or in combination, that reduce or abolish gene function.

There are a number of different strategies for creating conditional alleles of genes. Broadly, an allele can be conditional for function or expression. An example of an allele that is conditional for function is a temperature sensitive mutation where the gene product is functional at one temperature but non-functional at another, e.g., due to misfolding or mislocalization. One of ordinary skill in the art may produce mutant alleles which may have only one or a few altered nucleotides but which encode

inactive or temperature-sensitive proteins. Temperature-sensitive mutant yeast strains express a functional protein at permissive temperatures but do not express a functional protein at non-permissive temperatures.

An example of an allele that is conditional for expression is a chimeric gene where a regulated promoter controls the expression of the gene. Under one condition the gene is expressed and under another it is not. One may replace or alter the endogenous promoter of the gene with a heterologous or altered promoter that can be activated only under certain conditions. These conditional mutants only express the gene under defined experimental conditions. In a preferred embodiment, the gene is under the control of a regulated promoter where the gene may be expressed at higher or lower levels depending upon the degree of activation of the promoter. For instance, a gene under the control of a regulated promoter may be expressed at any level between 0 and 100% of wild type expression, such as at 10%, 20%, 50% or 80% of its wild type level. The gene may also be expressed at levels above its usual wild type expression (overexpression). All of these methods are well known in the art. For example, see Stark (1998), Garfinkel et al., (1998), and Lawrence and Rothstein, (1991), herein incorporated by reference.

One having ordinary skill in the art also may decrease expression of a gene without disrupting or mutating the gene. For instance, one may decrease the expression of a gene by transforming yeast with an antisense molecule or ribozyme under the control of a regulated or constitutive promoter (see Nasr et al., 1995, herein incorporated by reference). One may introduce an antisense construct operably linked to an inducible promoter into *S. cerevisiae* to study the function of a conditional allele (see Nasr et al. supra). One problem that may be encountered, however, is that many antisense molecules do not work well in yeast, for reasons that are, as yet, unclear (see Atkins et al., 1994 and Olsson et al., 1997).

One may also decrease gene expression by inserting a sequence by homologous recombination into or next to the gene of interest wherein the sequence targets the mRNA or the protein for degradation. For instance, one can introduce a construct that encodes ubiquitin such that a ubiquitin fusion protein is produced. This

protein will be likely to have a shorter half-life than the wildtype protein. See, e.g., Johnson et al. (1992), herein incorporated by reference.

In a preferred mode, a gene of interest is completely disrupted in order to ensure that there is no residual function of the gene. One can disrupt a gene by "classical" or PCR-based methods. The "classical" method of gene knockout is described by Rothstein (1991), herein incorporated by reference. However, it is preferable to use a PCR-based deletion method because it is faster and less labor intensive.

A preferred method to delete a gene is a one-step, polymerase chain reaction (PCR) based gene deletion method (Rothstein, 1991). Gene specific primer pairs are designed for PCR amplification of the plasmid pFA6a-KanMX4 (Wach et al., 1994), which teachings are herein incorporated by reference. The 3' ends of the upstream and downstream gene specific primers have been designed to include 18 basepairs (bp) and 19 bp, respectively, of nucleotide homology flanking the KanMX gene of the plasmid pFA6a-KanMX4 template. All of the gene specific primer pairs contain these complementary sequences, such that the same plasmid pFA6a-KanMX4 template can be used for all of the first round PCR reactions. At their 5' ends, the primers each have gene specific sequence homologies. The upstream primer contains a nucleotide sequence which includes the start codon of the gene to be knocked out and the sequence immediately upstream of the start codon. The downstream primer contains a nucleotide sequence which includes the stop codon of the gene and the sequence immediately downstream of the stop codon. For each set of primers, the sequences of the gene are derived from the 5' and 3' ends of the target DNA sequence.

The upstream and downstream primers are then used to amplify the pFA6a-KanMX4 by PCR using standard conditions for PCR. Hybridization conditions for specific gene-specific primers can be experimentally determined, or estimated by a number of formulas. One such formula is $T_m = 81.5 + 16.6 (\log_{10}[Na^+]) + 0.41$ (fraction G + C) - (600/N). See Sambrook et al. pages 11.46-11.47. The products of the first round PCR reactions are DNA molecules containing the KanMX marker

(conferring resistance to the drug G-418 in *S. cerevisiae*) flanked on both ends by 18 bp of gene specific sequences.

The gene specific flanking sequences are extended during the second round PCR reactions. The sequences of the two gene specific PCR primers are derived from the 45 bp immediately upstream (including the start codon) and the 45 bp immediately downstream (including the stop codon) of each gene. Thus, following the second round of PCR the product contains the KanMX marker flanked by 45 bp of gene specific sequences corresponding to the sequences flanking the gene's ORF. The PCR products are purified by an isopropanol precipitation, and shipped with the analytical primers (see below) to the consortium members on dry ice. The precipitated PCR products are resuspended in TE buffer (10 mM Tris-HCl [pH 7.6], 1 mM EDTA).

The various mutations are constructed in two related *Saccharomyces cerevisiae* strains, BY4741 (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) and BY4743 (*MATa/MATα his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0*) (Brachmann et al., 1998). Both of these strains are transformed with the PCR products by the lithium acetate method as described by Ito et al., 1983, and Schiestl and Gietz, 1989, herein incorporated by reference. The flanking, gene-specific yeast sequences target the integration event by homologous recombination to the desired locus (Figure 1). Transformants are selected on rich medium (YPD) which contains G-418 (Geneticin, Life Technologies, Inc.) as described by Guthrie and Fink, 1991, herein incorporated by reference. Ideally, independent mutations are isolated in the haploid (BY4741) and the diploid (BY4743) strains. The heterozygous mutant diploid strain is then sporulated, and subjected to tetrad analysis (Sherman, 1991; Sherman and Wakem, 1991, herein incorporated by reference). This allows for the isolation of the mutation in a *MATα* haploid strain. The two independently isolated *MATa* and *MATα* haploid strains are then mated to create a homozygous mutant diploid strain.

Methods to Characterize Yeast Gene Function

One of skill in the art will recognize that a number of methods can be used to characterize the function of a yeast gene. In general, the preferred strategy depends upon the assumptions made regarding the function of the gene. For example, if one creates a conditional allele of the gene, then one can engineer a mutant strain wherein the wildtype allele has been replaced by a conditional allele. See, e.g., Stark (1998). The strain is constructed and propagated under the permissive condition, and then the strain is switched to the non-permissive (or restrictive) condition and effects upon the cell's phenotype is monitored. This can be done in a haploid cell, or in a diploid cell as either a homozygous or heterozygous mutant.

A preferred method of characterizing the function of a gene is to knockout the gene completely and then analyze the knockout yeast strain by tetrad analysis. This method is preferred because one does not need to be able to engineer a conditional allele. Furthermore, as the knockout is a null allele, one is assured that it is the null phenotype that is assessed, rather than a phenotype resulting from a potentially hypomorphic conditional allele. In addition, a complete knockout of the gene can be constructed in a diploid strain where the potentially essential function of the gene is complemented by the second copy of the gene.

Once the knockout has been constructed as a heterozygous mutant, the effects of the mutation is assessed in the haploid spores. Tetrad analysis of the haploid spores allows for the genetic characterization of a mutation because one can determine the effect of the homozygous gene linked to the knockout marker (G-418 resistance).

Any of a number of different tests can be performed to determine the effect of knocking out the selected target gene. For instance, one can determine whether the yeast cell is more or less responsive to various pharmaceutical compounds (e.g., see Figure 4), pH, salinity, osmotic pressure, temperature or nutritional conditions. One can determine whether the knockout results in a different observable phenotype (e.g., see Figure 22). In addition, yeast cells can be tested for their ability to mate, sporulate and bud relative to a wild type control. Thus, these tests may provide important information regarding the function of the target gene.

Methods to Identify Potential Homologs in Other Organisms

Once a gene has been identified as a potential target, one can determine whether the gene from yeast has homologs in other organisms, such as humans, non-human mammals, other vertebrates such as fish, insects, plants, or other fungi.

One method of determining whether an *S. cerevisiae* gene has homologs is by the use of low stringency hybridization and washing. In general, genomic DNA or cDNA libraries can be screened using probes derived from the target *S. cerevisiae* gene using methods known in the art. See above and pages 8.46-8.49 and 9.46-9.58 of Sambrook et al., 1989, herein incorporated by reference. Preferably, genomic DNA libraries are screened because cDNA libraries generally will not contain all the mRNA species an organism can make. Genomic DNA libraries from a variety of different organisms, such as plants, fungi, insects, and various mammalian species are commercially available and can be screened. This method is useful for determining whether there are homologs in organisms whose DNA sequences have not been characterized extensively.

A second method of determining whether an *S. cerevisiae* gene has homologs is through the use of degenerate PCR. In this method, degenerate oligonucleotides that encode short amino acid sequences of the *S. cerevisiae* gene are made. Methods of preparing degenerate oligonucleotides and using them in PCR to isolate uncloned genes are well known in the art (see Sambrook, pages 14.7-14.8, and Crawley et al., 1997, pages 4.2.1-4.2.5, herein incorporated by reference).

The most preferred method is to compare the sequence of the *S. cerevisiae* gene to sequences from other organism. Either the nucleotide sequence of the gene or its encoded amino acid sequence is compared to the sequences from other organisms. Preferably, the encoded amino acid sequence of the yeast gene is compared to amino acid sequences from other organisms. The sequence of the yeast gene can be compared by a number of different algorithms well known in the art. In general, computer programs designed for sequence analysis are used for the purpose of comparing the sequence of interest to a large database of other sequences. Any computer program designed for the purpose of sequence comparison can be used in

this method. Some computer programs, such as Fasta, produce results that are typically presented as "% sequence identity." Other computer programs, such as blastp, produce results presented as "p-values." Preferably, the target gene sequence will be compared to other sequences using the blastp algorithm.

Nucleotide and amino acid sequences of target genes may be compared to vertebrate sequences, including human and non-human mammalian sequences, as well as plant and insect sequences using any one of the large number of programs known in the art for comparing nucleotide and amino acid sequences to sequences in a database. Examples of such programs are Fasta and blastp, discussed above. Examples of databases which can be searched include GenBank-EMBL, SwissProt, DDBJ, GeneSeq, and EST databases, as well as databases containing combinations of these databases.

As a further characterization, any potential homologs from other organisms can be assessed for their ability to functionally complement the yeast mutant. This can be achieved by first cloning the homolog into a *S. cerevisiae* expression vector by standard methods. This plasmid can then be transformed into the heterozygous mutant diploid strain. Upon sporulation and tetrad dissection the ability of the homolog to complement the yeast function is determined by whether or not the haploid spores complements the yeast knockout and restores the wildtype function of the haploid spore. The ability of the homolog to complement the yeast mutant would indicate shared function(s) and suggest that the homolog may be part of a similar pathway in the other organism.

Nucleic Acids, Vectors and Production of Recombinant Polypeptides

The present invention provides nucleic acids and recombinant DNA vectors which comprise *S. cerevisiae* RIG and target gene DNA sequences. Specifically, vectors comprising all or portions of the DNA sequence of *HES1*, *YMR134w*, *YER034w*, *YJL105w*, *YKL077w*, *YGR046w*, *YJR041c*, *YER044c* and *YLR100w* are provided. The vectors of this invention also include those comprising DNA sequences which hybridize under stringent conditions to the *HES1*, *YMR134w*,

YER034w, *YJL105w*, *YKL077w*, *YGR046w*, *YJR041c*, *YER044c* and *YLR100w* gene sequences, and conservatively modified variations thereof.

The nucleic acids of this invention include single-stranded and double-stranded DNA, RNA, oligonucleotides, antisense molecules, or hybrids thereof and may be isolated from biological sources or synthesized chemically or by recombinant DNA methodology. The nucleic acids, recombinant DNA molecules and vectors of this invention may be present in transformed or transfected cells, cell lysates, or in partially purified or substantially pure forms.

DNA sequences may be expressed by operatively linking them to an expression control sequence in an appropriate expression vector and employing that expression vector to transform an appropriate unicellular host. Expression control sequences are sequences which control the transcription, post-transcriptional events and translation of DNA sequences. Such operative linking of a DNA sequence of this invention to an expression control sequence, of course, includes, if not already part of the DNA sequence, the provision of a translation initiation codon, ATG, in the correct reading frame upstream of the DNA sequence.

A wide variety of host/expression vector combinations may be employed in expressing the DNA sequences of this invention. Useful expression vectors, for example, may consist of segments of chromosomal, non-chromosomal and synthetic DNA sequences.

Useful expression vectors for bacterial hosts include bacterial plasmids, such as those from *E. coli*, including pBluescript, pGEX-2T, pUC vectors, col E1, pCR1, pBR322, pMB9 and their derivatives, wider host range plasmids, such as RP4, phage DNAs, e.g., the numerous derivatives of phage lambda, e.g., NM989, λGT10 and λGT11, and other phages, e.g., M13 and filamentous single stranded phage DNA. In yeast, vectors include Yeast Integrating plasmids (*e.g.*, YIp5) and Yeast Replicating plasmids (the YRp and YEp series plasmids), Yeast centromere plasmids (the YCp series plasmids), pGPD-2, 2μ plasmids and derivatives thereof, and improved shuttle vectors such as those described in Gietz and Sugino, Gene, 74, pp. 527-34 (1988) (YIplac, YEplac and YCplac). Expression in mammalian cells can be achieved using a

variety of plasmids, including pSV2, pBC12BI, and p91023, as well as lytic virus vectors (*e.g.*, vaccinia virus, adeno virus, and baculovirus), episomal virus vectors (*e.g.*, bovine papillomavirus), and retroviral vectors (*e.g.*, murine retroviruses). Useful vectors for insect cells include baculoviral vectors and pVL 941.

In addition, any of a wide variety of expression control sequences -- sequences that control the expression of a DNA sequence when operatively linked to it -- may be used in these vectors to express the DNA sequences of this invention. Such useful expression control sequences include the expression control sequences associated with structural genes of the foregoing expression vectors. Expression control sequences that control transcription include, e.g., promoters, enhancers and transcription termination sites. Expression control sequences that control post-transcriptional events include splice donor and acceptor sites and sequences that modify the half-life of the transcribed RNA, e.g., sequences that direct poly(A) addition or binding sites for RNA-binding proteins. Expression control sequences that control translation include ribosome binding sites, sequences which direct expression of the polypeptide to particular cellular compartments, and sequences in the 5' and 3' untranslated regions that modify the rate or efficiency of translation.

Examples of useful expression control sequences include, for example, the early and late promoters of SV40 or adenovirus, the lac system, the trp system, the TAC or TRC system, the T3 and T7 promoters, the major operator and promoter regions of phage lambda, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, e.g., Pho5, the promoters of the yeast α-mating system, the GAL1 or GAL10 promoters, and other constitutive and inducible promoter sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof. See, e.g., The Molecular Biology of the Yeast Saccharomyces (eds. Strathern, Jones and Broach) Cold Spring Harbor Lab., Cold Spring Harbor, N.Y. for details on yeast molecular biology in general and on yeast expression systems (pp. 181-209) (incorporated herein by reference)).

DNA vector design for transfection into mammalian cells should include appropriate sequences to promote expression of the gene of interest, including: appropriate transcription initiation, termination and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion. A great number of expression control sequences -- constitutive, inducible and/or tissue-specific -- are known in the art and may be utilized. For eukaryotic cells, expression control sequences typically include a promoter, an enhancer derived from immunoglobulin genes, SV40, cytomegalovirus, etc., and a polyadenylation sequence which may include splice donor and acceptor sites. Substantial progress in the development of mammalian cell expression systems has been made in the last decade and many aspects of the system are well characterized.

Preferred DNA vectors also include a marker gene and means for amplifying the copy number of the gene of interest. DNA vectors may also comprise stabilizing sequences (e.g., ori- or ARS-like sequences and telomere-like sequences), or may alternatively be designed to favor directed or non-directed integration into the host cell genome. In a preferred embodiment, DNA sequences of this invention are inserted in frame into an expression vector that allows high level expression of an RNA which encodes a fusion protein comprising encoded DNA sequence of interest.

Of course, not all vectors and expression control sequences will function equally well to express the DNA sequences of this invention. Neither will all hosts function equally well with the same expression system. However, one of skill in the art may make a selection among these vectors, expression control sequences and hosts without undue experimentation and without departing from the scope of this invention. For example, in selecting a vector, the host must be considered because the vector must be replicated in it. The vector's copy number, the ability to control that copy number, the ability to control integration, if any, and the expression of any other

proteins encoded by the vector, such as antibiotic or other selection markers, should also be considered.

In selecting an expression control sequence, a variety of factors should also be considered. These include, for example, the relative strength of the sequence, its controllability, and its compatibility with the DNA sequence of this invention, particularly with regard to potential secondary structures. Unicellular hosts should be selected by consideration of their compatibility with the chosen vector, the toxicity of the product coded for by the DNA sequences of this invention, their secretion characteristics, their ability to fold the polypeptide correctly, their fermentation or culture requirements, and the ease of purification from them of the products coded for by the DNA sequences of this invention.

Within these parameters, one of skill in the art may select various vector/expression control sequence/host combinations that will express the DNA sequences of this invention in fermentation or in other large scale cultures.

Given the strategies described herein, one of skill in the art can construct a variety of vectors and nucleic acid molecules comprising functionally equivalent nucleic acids. DNA cloning and sequencing methods are well known to those of skill in the art and are described in an assortment of laboratory manuals, including Sambrook et al, supra, 1989; and Ausubel et al., 1994 Supplement. Product information from manufacturers of biological, chemical and immunological reagents also provide useful information.

The recombinant DNA molecules and more particularly, the expression vectors of this invention may be used to express the RIG and target genes from *S. cerevisiae* as recombinant polypeptides in a heterologous host cell. The polypeptides of this invention may be full-length or less than full-length polypeptide fragments recombinantly expressed from the DNA sequences according to this invention. Such polypeptides include variants and muteins having biological activity. The polypeptides of this invention may be soluble, or may be engineered to be membrane- or substrate-bound using techniques well known in the art.

Particular details of the transfection, expression and purification of recombinant proteins are well documented and are understood by those of skill in the art. Further details on the various technical aspects of each of the steps used in recombinant production of foreign genes in mammalian cell expression systems can be found in a number of texts and laboratory manuals in the art. See, e.g., Ausubel et al., 1989, herein incorporated by reference.

Transformation and other methods of introducing nucleic acids into a host cell (e.g., transfection, electroporation, liposome delivery, membrane fusion techniques, high velocity DNA-coated pellets, viral infection and protoplast fusion) can be accomplished by a variety of methods which are well known in the art (see, for instance, Ausubel, *supra*, and Sambrook, *supra*). Bacterial, yeast, plant or mammalian cells are transformed or transfected with an expression vector, such as a plasmid, a cosmid, or the like, wherein the expression vector comprises the DNA of interest. Alternatively, the cells may be infected by a viral expression vector comprising the DNA or RNA of interest. Depending upon the host cell, vector, and method of transformation used, transient or stable expression of the polypeptide will be constitutive or inducible. One having ordinary skill in the art will be able to decide whether to express a polypeptide transiently or stably, and whether to express the protein constitutively or inducibly.

A wide variety of unicellular host cells are useful in expressing the DNA sequences of this invention. These hosts may include well known eukaryotic and prokaryotic hosts, such as strains of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, fungi, yeast, insect cells such as *Spodoptera frugiperda* (SF9), animal cells such as CHO, BHK, MDCK and various murine cells, e.g., 3T3 and WEHI cells, African green monkey cells such as COS 1, COS 7, BSC 1, BSC 40, and BMT 10, and human cells such as VERO, WI38, and HeLa cells, as well as plant cells in tissue culture.

Expression of recombinant DNA molecules according to this invention may involve post-translational modification of a resultant polypeptide by the host cell. For example, in mammalian cells expression might include, among other things, glycosylation, lipidation or phosphorylation of a polypeptide, or cleavage of a signal

sequence to produce a "mature" protein. Accordingly, the polypeptide expression products of this invention encompass full-length polypeptides and modifications or derivatives thereof, such as glycosylated versions of such polypeptides, mature proteins and polypeptides retaining a signal peptide. The present invention also provides for biologically active fragments of the polypeptides. Sequence analysis or genetic manipulation may identify those domains responsible for the function of the protein in yeast. Thus, the invention encompasses the production of biologically active fragments. The invention also encompasses fragments of the polypeptides which would be valuable as antigens for the production of antibodies, or as competitors for antibody binding.

The polypeptides of this invention may be fused to other molecules, such as genetic, enzymatic or chemical or immunological markers such as epitope tags. Fusion partners include, *inter alia*, *myc*, hemagglutinin (HA), GST, immunoglobulins, β-galactosidase, biotin trpE, protein A, β-lactamase, α amylase, maltose binding protein, alcohol dehydrogenase, polyhistidine (for example, six histidine at the amino and/or carboxyl terminus of the polypeptide), lacZ, green fluorescent protein (GFP), yeast α mating factor, GAL4 transcription activation or DNA binding domain, luciferase, and serum proteins such as ovalbumin, albumin and the constant domain of IgG. See, e.g., Godowski et al., 1988, and Ausubel et al., *supra*. Fusion proteins may also contain sites for specific enzymatic cleavage, such as a site that is recognized by enzymes such as Factor XIII, trypsin, pepsin, or any other enzyme known in the art. Fusion proteins will typically be made by either recombinant nucleic acid methods, as described above, chemically synthesized using techniques such as those described in Merrifield, 1963, herein incorporated by reference, or produced by chemical cross-linking.

Tagged fusion proteins permit easy localization, screening and specific binding via the epitope or enzyme tag. See Ausubel, 1991, Chapter 16. Some tags allow the protein of interest to be displayed on the surface of a phagemid, such as M13, which is useful for panning agents that may bind to the desired protein targets.

Thus, fusion proteins are useful for screening potential agents using the proteins encoded by the target genes.

One advantage of fusion proteins is that an epitope or enzyme tag can simplify purification. These fusion proteins may be purified, often in a single step, by affinity chromatography. For example, a His^6 tagged protein can be purified on a Ni affinity column and a GST fusion protein can be purified on a glutathione affinity column. Similarly, a fusion protein comprising the Fc domain of IgG can be purified on a Protein A or Protein G column and a fusion protein comprising an epitope tag such as myc can be purified using an immunoaffinity column containing an anti-c-myc antibody. It is preferable that the epitope tag be separated from the protein encoded by the target gene by an enzymatic cleavage site that can be cleaved after purification. A second advantage of fusion proteins is that the epitope tag can be used to bind the fusion protein to a plate or column through an affinity linkage for screening targets.

In addition, fusion proteins comprising the constant domain of IgG or other serum proteins can increase a protein's half-life in circulation for use therapeutically. Fusion proteins comprising a targeting domain can be used to direct the protein to a particular cellular compartment or tissue target in order to increase the efficacy of the functional domain. See, e.g., U.S. Pat. No. 5,668,255, which discloses a fusion protein containing a domain which binds to an animal cell coupled to a translocation domain of a toxin protein. Fusion proteins may also be useful for improving antigenicity of a protein target. Examples of making and using fusion proteins are found in U.S. Pat. Nos. 5,225,538, 5,821,047, and 5,783,398, which are hereby incorporated by reference.

Production of Polypeptide Fragments, Derivatives and Muteins and Biological Assays Thereof

Fragments, derivatives and muteins of polypeptides encoded by the RIG and target genes can be produced recombinantly or chemically, as discussed above. One can produce fragments of a polypeptide encoding a target gene by truncating the DNA encoding the target gene and then expressing it recombinantly. Alternatively,

one can produce a fragment by chemically synthesizing a portion of the full-length polypeptide. One may also produce a fragment by enzymatically cleaving the polypeptide. Methods of producing polypeptide fragments are well-known in the art (see, e.g., Sambrook et al. and Ausubel et al. *supra*).

One may produce muteins of a polypeptide encoded by a target gene by introducing mutations into the DNA sequence of the gene and then expressing it recombinantly. These mutations may be targeted, in which particular encoded amino acids are altered, or may be untargeted, in which random encoded amino acids within the polypeptide are altered. Muteins with random amino acid alterations can be screened for a particular biological activity. Methods of producing muteins with targeted or random amino acid alterations are well known in the art, see e.g., Sambrook et al., Ausubel et al., *supra,* and U.S. Pat. No. 5,223,408, herein incorporated by reference. Production of polypeptide derivatives are well known in the art, see above.

There are a number of methods known in the art to determine whether fragments, muteins and derivatives of polypeptides encoded by a target gene has the same, enhanced or decreased biological activity as the wild type polypeptide. One of the simplest assays involves determining whether the fragment, mutein or derivative can complement the gene function in a cell which does not contain the target gene. For instance, one can introduce a DNA encoding a fragment or mutein of a polypeptide encoded by a gene into a mutant yeast strain which has the gene of interest deleted (see above under "Methods of Producing Mutant Yeast Strains"). If introduction of the DNA encoding the fragment or mutein permits the mutant yeast strain to regain its wildtype phenotype, then the fragment or mutein is biologically active, and complements the deleted gene.

In one type of screening assay, the target gene or a fragment thereof can be used as the "bait" in a two-hybrid screen to identify molecules that physically interact with the target gene. See Chien et al. (1991).

In addition, one may generate genome expression profiles of yeast strains to characterize the gene's function. In order to generate such profiles, a non-

functional or conditional allele of the gene in a yeast strain must be produced. The conditional or non-functional allele may be constructed by any technique known in the art, including deleting the gene as described above, making a temperature-sensitive allele of the gene or operably linking the gene to an inducible promoter for regulated expression. If the yeast strain contains a non-functional allele, a genome expression profile of the mutant strain is compared to a wild type strain. If the yeast strain contains a conditional allele, the yeast strain is first grown under the permissive condition to permit expression of the functional product of the targetl gene. Then, the yeast strain is shifted to the nonpermissive condition, in which the product of the target gene is not made or is non-functional. The genome expression profile of the yeast strain under the nonpermissive condition may be compared to the same yeast strain grown under permissive conditions or a wildtype yeast strain. Structure-function studies can be performed wherein a library of mutant forms of the gene is screened for the ability to complement the knock-out mutant strain.

Fragments, muteins and derivatives may also be micro-injected into a mutant yeast strain in which the gene of interest is deleted to determine whether the introduction of the fragment, mutein or derivative can complement the genetic defect. Similarly, fragments, muteins and derivatives may be microinjected into other cell types in which the homologous gene has been deleted.

Finally, if a particular biochemical activity of a polypeptide encoded by a target gene is known, this activity can be measured for fragments, muteins or derivatives of the polypeptide. For instance, if a target gene encodes a kinase, one could measure the kinase activity of the wild type polypeptide and compare it to the activity of a fragment, mutein or derivative.

Production of Antibodies

The polypeptides encoded by the target genes of this invention may be used to elicit polyclonal or monoclonal antibodies which bind to the target gene product or a homolog from another species using a variety of techniques well known to those of skill in the art. Alternatively, peptides corresponding to specific regions of

the polypeptide encoded by the target gene may be synthesized and used to create immunological reagents according to well known methods.

Antibodies directed against the polypeptides of this invention are immunoglobulin molecules or portions thereof that are immunologically reactive with the polypeptide of the present invention. It should be understood that the antibodies of this invention include antibodies immunologically reactive with fusion proteins.

Antibodies directed against a polypeptide encoded by a target gene may be generated by immunization of a mammalian host. Such antibodies may be polyclonal or monoclonal. Preferably they are monoclonal. Methods to produce polyclonal and monoclonal antibodies are well known to those of skill in the art. For a review of such methods, see Harlow and Lane (1988), Yelton et al. (1981), and Ausubel et al. (1989) herein incorporated by reference. Determination of immunoreactivity with a polypeptide encoded by an target gene may be made by any of several methods well known in the art, including by immunoblot assay and ELISA.

Monoclonal antibodies with affinities of 10^{-8} M^{-1} or preferably 10^{-9} to 10^{-10} M^{-1} or stronger are typically made by standard procedures as described, e.g., in Harlow and Lane, 1988 or Goding, 1986. Briefly, appropriate animals are selected and the desired immunization protocol followed. After the appropriate period of time, the spleens of such animals are excised and individual spleen cells fused, typically, to immortalized myeloma cells under appropriate selection conditions. Thereafter, the cells are clonally separated and the supernatants of each clone tested for their production of an appropriate antibody specific for the desired region of the antigen.

Other suitable techniques involve *in vitro* exposure of lymphocytes to the antigenic polypeptides, or alternatively, to selection of libraries of antibodies in phage or similar vectors. See Huse et al., 1989. The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, polypeptides and antibodies will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes,

substrates, cofactors, inhibitors, fluorescent agents, chemiluminescent agents, magnetic particles and the like. Patents teaching the use of such labels include U.S. Patents 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149 and 4,366,241, herein incorporated by reference. Also, recombinant immunoglobulins may be produced (see U.S. Patent 4,816,567, herein incorporated by reference).

An antibody of this invention may also be a hybrid molecule formed from immunoglobulin sequences from different species (e.g., mouse and human) or from portions of immunoglobulin light and heavy chain sequences from the same species. An antibody may be a single-chain antibody or a humanized antibody. It may be a molecule that has multiple binding specificities, such as a bifunctional antibody prepared by any one of a number of techniques known to those of skill in the art including the production of hybrid hybridomas, disulfide exchange, chemical cross-linking, addition of peptide linkers between two monoclonal antibodies, the introduction of two sets of immunoglobulin heavy and light chains into a particular cell line, and so forth.

The antibodies of this invention may also be human monoclonal antibodies, for example those produced by immortalized human cells, by SCID-hu mice or other non-human animals capable of producing "human" antibodies, or by the expression of cloned human immunoglobulin genes. The preparation of humanized antibodies is taught by U.S. Pat. Nos. 5,777,085 and 5,789,554, herein incorporated by reference.

In sum, one of skill in the art, provided with the teachings of this invention, has available a variety of methods which may be used to alter the biological properties of the antibodies of this invention including methods which would increase or decrease the stability or half-life, immunogenicity, toxicity, affinity or yield of a given antibody molecule, or to alter it in any other way that may render it more suitable for a particular application.

Therapeutic Methods Using Nucleic Acids Encoding Target Genes

Once a target gene has been identified in *S. cerevisiae*, the gene and its nucleotide sequence can be exploited in a number of ways depending upon the nature of the target gene. One method is to use the primary sequence of the target gene itself. For instance, antisense oligonucleotides can be produced which are complementary to the mRNA of the target gene. Antisense oligonucleotides can be used to inhibit transcription or translation of a target yeast gene. Production of antisense oligonucleotides effective for therapeutic use is well-known in the art, see Agrawal et al., 1998, Lavrovsky et al., 1997, and Crooke, 1998, herein incorporated by reference. Antisense oligonucleotides are often produced using derivatized or modified nucleotides in order to increase half-life or bioavailability.

The primary sequence of the target gene can also be used to design ribozymes that can target and cleave specific target gene sequences. There are a number of different types of ribozymes. Most synthetic ribozymes are generally hammerhead, *Tetrahymena* and hairpin ribozymes. Methods of designing and using ribozymes to cleave specific RNA species are known in the art, see Zhao et al., 1998, Larovsky et al., 1997, and Eckstein, 1997, herein incorporated by reference. Although hammerhead ribozymes are generally ineffective in yeast (Castanotto et al., 1998), other types of ribozymes may be effective in yeast, and hammerhead and other types of ribozymes are effective in other organisms.

As discussed above, one can use target yeast genes to identify homologous genes in plants and animals, including humans. Therefore, one can design ribozymes and antisense molecules to these genes from plants and animals, including humans.

Methods Using Neutralizing Antibodies to Proteins Encoded by Target Genes

The protein encoded by the target gene can be used to elicit neutralizing antibodies for use as inhibit the function of the target protein. An antibody may be an especially good inhibitor if the target gene of interest encodes a protein which is expressed on the cell surface, such as an integral membrane protein. Although

polyclonal antibodies may be made, monoclonal antibodies are preferred. Monoclonal antibodies can be screened individually in order to isolate those that are neutralizing or inhibitory for the protein encoded by the target gene. Monoclonal antibodies also may be screened for inhibition of a particular function of a protein. For instance, if it is known that the target gene in yeast encodes an enzyme, one can identify antibodies that inhibit the enzymatic activity. Alternatively, if the specific function of a target gene is unknown, one can measure inhibition of the protein by determining the genome expression profile for yeast cells contacted with the neutralizing antibody. Similarly, one can screen antibodies which are directed against animal, plant or human proteins for inhibition of the protein's activity in appropriate cells.

Monoclonal antibodies which inhibit a target protein *in vitro* may be humanized for therapeutic use using methods well-known in the art, see, e.g., U.S. Pat. Nos. 5,777,085 and 5,789,554, herein incorporated by reference. Monoclonal antibodies may also be engineered as single-chain antibodies using methods well-known in the art for therapeutic use, see, e.g., U.S. Pat. Nos. 5,091,513, 5,587,418, and 5,608,039, herein incorporated by reference.

Neutralizing antibodies may also be used diagnostically. For instance, the binding site of a neutralizing antibody to the protein encoded by the target gene can be used to help identify domains that are required for the protein's activity. The information about the critical domains of a target protein can be used to design inhibitors that bind to the critical domains of the target protein. In addition, neutralizing antibodies can be used to validate whether a potential inhibitor of an target protein inhibits the protein in *in vitro* assays.

Methods of Identifying Functional Attributes of the Target

Once a target gene in yeast is identified, the GRM (or an equivalent) is used to help identify critical functional attributes of the gene. In order to determine the particular transcripts a target gene modifies, one overexpresses the target gene in the cells of the GRM. One may also overexpress a conditional allele of the gene in the cells of the GRM. Then, one identifies a subset of genes that are either induced or

repressed by overexpression of the target gene. Methods for processing data using the GRM are also disclosed in United States Patents 5,569,588 and 5,777,888; see also United States Patent Application Serial No. 09/076,668, now pending. Once the genes that are regulated by a target gene are identified, one can use this information in a number of ways to identify potential inhibitors or activators of the target protein. Alternatively, one may determine the genome expression profile of a cell that has a mutation in a target gene, or a cell that has the endogenous target gene replaced either with an altered allele or with the counterpart gene from another species. Similarly, plant and animal GRMs, including human GRMs, overexpressing target genes can be used in the same way to identify potential inhibitors or activators of the target protein in these organisms.

Another method for isolating a potential inhibitors or activators of a target gene is to use information obtained from the "two-hybrid system" to identify and clone genes encoding proteins that interact with the polypeptide encoded by the target gene (see, e.g., Chien et al.,1991, incorporated herein by reference). The amino acid sequences of the polypeptides identified by the two-hybrid system can be used to design inhibitory peptides to the target protein. The "two-hybrid" system using libraries of the appropriate species can also be used to identify and clone genes encoding proteins that interact with the polypeptide encoded by the target genes.

Methods of Using Target Proteins

Recombinantly expressed target proteins or functional fragments thereof can be used to screen libraries of natural, semisynthetic or synthetic compounds. Particularly useful types of libraries include combinatorial small organic molecule libraries, phage display libraries, and combinatorial peptide libraries. Methods of determining whether components of the library bind to a particular polypeptide are well known in the art. In general, the polypeptide target is attached to solid support surface by non-specific or specific binding. Specific binding can be accomplished using an antibody which recognizes the protein that is bound to a solid support, such as a plate or column. Alternatively, specific binding may be through an

epitope tag, such as GST binding to a glutathione-coated solid support, or IgG fusion protein binding to a Protein A solid support. Alternatively, the recombinantly expressed protein or fragments thereof may be expressed on the surface of phage, such as M13. A library in mobile phase is incubated under conditions to promote specific binding between the target and a compound. Compounds which bind to the target can then be identified. Alternately, the library is attached to a solid support and the polypeptide target is in the mobile phase.

Binding between a compound and target can be determined by a number of methods. The binding can be identified by such techniques as competitive ELISAs or RIAs, for example, wherein the binding of a compound to a target will prevent an antibody to the target from binding. These methods are well-known in the art, see, e.g., Harlow and Lane, *supra*. Another method is to use BiaCORE (BiaCORE) to measure interactions between a target and a compound using methods provided by the manufacturer. A preferred method is automated high throughput screening, see, e.g., Burbaum et al., 1997, and Schullek et al., 1997, herein incorporated by reference.

Once a compound that binds to a target is identified, one then determines whether the compound inhibits the activity of the target. If a biological function for the target protein is known, one could determine whether the compound inhibited the biological activity of the protein. For instance, if it is known that the target protein is an enzyme, one can measure the inhibition of enzymatic activity in the presence of the potential inhibitor.

In a preferred embodiment, the target gene is selected from *YMR134w*, *YER034w*, *YJL105w*, *YKL077w*, *YGR046w*, *YJR041c*, *YER044c* and *YLR100w* and their mammalian homologs.

Another embodiment of the invention is to use the recombinantly expressed protein for rational drug design. The structure of the recombinant protein may be determined using x-ray crystallography or nuclear magnetic resonance (NMR). Alternatively, one could use computer modeling to determine the structure of the protein. The structure can be used in rational drug design to design potential inhibitory

compounds of the target (see, e.g., Clackson, Mattos et al., Hubbard, Cunningham et al., Kubinyi, Kleinberg et al., all herein incorporated by reference).

In another embodiment, potential inhibitors of a regulon target gene can be identified by the following steps:

a) creating a host cell in which the target gene has been altered or inactivated by mutation;

b) comparing gene expression profiles in the mutated host cell to those in a host cell which expresses the normal target gene;

c) identifying one or more potential target-dependent reporter genes whose expression is altered in the host cell in which the target gene has been altered or inactivated compared to the host cell which expresses the normal target gene; and

d) screening one or more compounds for their effects on expression of the target-dependent reporter gene.

If expression of the target-dependent reporter gene increases in the host cell harboring an altered or inactivated target gene, then a potential inhibitor of the regulon target gene will increase expression of the target-dependent reporter gene, and if expression of the target-dependent reporter gene decreases in the host cell harboring an altered or inactivated target gene, then a potential inhibitor of the regulon target gene will decrease expression of the target-dependent reporter gene.

The method may further comprise the step, performed before step d), of assessing the specificity of a potential target-dependent reporter gene by comparing gene expression profiles the potential target-dependent reporter gene to a plurality of genes in a database of compiled gene expression profiles to generate individual expression correlation coefficients wherein a target-dependent reporter gene whose expression correlates with the expression of the regulon target gene and with a minimal number or no other gene is selected over one whose expression correlates with a greater number of genes based on expression correlation coefficients. The method may also encompass upstream sequences that control expression of the target-dependent reporter genes fused to a heterologous coding sequence, and the fusion is

used to screen compounds for potential inhibitors of the regulon target gene, as discussed above.

In a preferred embodiment, the target gene is selected from *YMR134w*, *YER034w*, *YJL105w*, *YKL077w*, *YGR046w*, *YJR041c*, *YER044c* and *YLR100w* and their mammalian homologs.

Pharmaceutical Applications

Compounds that bind to target proteins or regulate target gene expression can be tested in yeast cell systems and heterologous host cell systems (e.g., human cells) to verify that they do not have undesirable side effects. In addition, the yeast GRM can be used to make sure that the compounds do not adversely alter gene transcription (e.g., in an undesirable way). Of course, certain changes in gene expression may be inevitable and many of these will not be deleterious to the patient or host organism. Once lead compounds have been identified, these compounds can be refined further via rational drug design and other standard pharmaceutical techniques.

The compounds of this invention may be formulated into pharmaceutical compositions and administered *in vivo* at an effective dose to treat a particular disease or condition. Determination of a preferred pharmaceutical formulation and a therapeutically efficient dose regiment for a given application is within the skill of the art taking into consideration, for example, the condition and weight of the patient, the extent of desired treatment and the tolerance of the patient for the treatment.

Administration of the compounds of this invention, including isolated and purified forms, their salts or pharmaceutically acceptable derivatives thereof, may be accomplished using any conventionally accepted mode of administration.

The pharmaceutical compositions of this invention may be in a variety of forms, which may be selected according to the preferred modes of administration. These include, for example, solid, semi-solid and liquid dosage forms such as tablets, pills, powders, liquid solutions or suspensions, suppositories, and injectable and infusible solutions. The preferred form depends on the intended mode of

administration and therapeutic application. Modes of administration may include oral, parenteral, subcutaneous, intravenous, intralesional or topical administration.

The compounds of this invention may, for example, be placed into sterile, isotonic formulations with or without cofactors which stimulate uptake or stability. The formulation is preferably liquid, or may be lyophilized powder. For example, the inhibitors may be diluted with a formulation buffer comprising 5.0 mg/ml citric acid monohydrate, 2.7 mg/ml trisodium citrate, 41 mg/ml mannitol, 1 mg/ml glycine and 1 mg/ml polysorbate 20. This solution can be lyophilized, stored under refrigeration and reconstituted prior to administration with sterile Water-For-Injection (USP).

Topical administration includes administration to the skin or mucosa, including surfaces of the lung and eye. Compositions for topical administration, including those for inhalation, may be prepared as a dry powder which may be pressurized or non-pressurized. In non-pressurized powder compositions, the active ingredient in finely divided form may be used in admixture with a larger-sized pharmaceutically acceptable inert carrier comprising particles having a size, for example, of up to 100 micrometers in diameter. Alternatively, the composition may be pressurized and contain a compressed gas, such as nitrogen or a liquified gas propellant. The liquified propellant medium and indeed the total composition is preferably such that the active ingredient does not dissolve therein to any substantial extent.

Dosage forms for topical or transdermal administration of a compound of this invention include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants or patches. The active component is admixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives or buffers as may be required. Ophthalmic formulation, ear drops, eye ointments, powders and solutions are also contemplated as being within the scope of this invention.

The pharmaceutical compositions of this invention may also be administered using microspheres, microparticulate delivery systems or other sustained release formulations placed in, near, or otherwise in communication with affected

tissues or the bloodstream. Suitable examples of sustained release carriers include semipermeable polymer matrices in the form of shaped articles such as suppositories or microcapsules. Implantable or microcapsular sustained release matrices include polylactides (U.S. Patent No. 3,773,319; EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al., 1985); poly(2-hydroxyethyl-methacrylate) or ethylene vinyl acetate (Langer et al., 1981, Langer, 1982).

The compounds of this invention may also be attached to liposomes, which may optionally contain other agents to aid in targeting or administration of the compositions to the desired treatment site. Attachment of the compounds to liposomes may be accomplished by any known cross-linking agent such as heterobifunctional cross-linking agents that have been widely used to couple toxins or chemotherapeutic agents to antibodies for targeted delivery. Conjugation to liposomes can also be accomplished using the carbohydrate-directed cross-linking reagent 4-(4-maleimidophenyl) butyric acid hydrazide (MPBH) (Duzgunes et al., 1992), herein incorporated by reference.

Liposomes containing pharmaceutical compounds may be prepared by well-known methods (See, e.g. DE 3,218,121; Epstein et al., 1985; Hwang et al.,1980; U.S. Patent Nos. 4,485,045 and 4,544,545). Ordinarily the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol.% cholesterol. The proportion of cholesterol is selected to control the optimal rate of MAG derivative and inhibitor release.

The compositions also will preferably include conventional pharmaceutically acceptable carriers well known in the art (see, e.g., Remington's Pharmaceutical Sciences, 16th Edition, 1980, Mac Publishing Company). Such pharmaceutically acceptable carriers may include other medicinal agents, carriers, genetic carriers, adjuvants, excipients, etc., such as human serum albumin or plasma preparations. The compositions are preferably in the form of a unit dose and will usually be administered one or more times a day.

EXAMPLE 1: PREPARATION OF THE Genome Reporter Matrix™

Construction of Reporter Gene Fusions (Method 1)

The regulatory region of each yeast gene was cloned into one of two vectors, pAB1 or pAB2. The vector pAB1 was constructed in the following manner: First, the polymerase chain reaction (PCR) was used to amplify the transcriptional terminator region from the gene PGK1 using the oligonucleotides 5P-PGKTERM (5'-GATTGAATTCAATTGAAATCGATAG-3') and 3P-PGKTERM (5'-CCGAGGCGCCGAATTTTCGAGTTAT-3'). The amplified fragment consists of the 263 base-pair region immediately downstream of the PGK1 stop codon, and contains an EcoRI site at the 5' end and a NarI site at the 3' end. These restriction sites were engineered into the two PCR primers (underlined sequences). The terminator was then cloned into YIplac211 that had been linearized with EcoRI and NarI, yielding pAB34. Next, the coding region of the green fluorescent protein (GFP) from *Aequoria victoria* was amplified by PCR using the oligonucleotides 5P-GFP-ORF (5'-CATGTCTAGAGGAGAAGAACTTTTC-3') and 3P-GFP-ORF (5'-CGCGAATTCCTATTTGTATAGTTCA-3'). Again, these oligonucleotides contain engineered XbaI and EcoRI sites at the 5' and 3' ends, respectively (underlined). This fragment was cloned into pAB34, linearized with XbaI and EcoRI, to produce pAB35. Finally, the GFP-PGK terminator fragment was moved into the episomal vector YEplac195 (9) as an XbaI/NarI fragment, thereby producing pAB1.

The vector pAB2 is pAB1 with an altered multiple cloning site (MCS). The new MCS contains 8 basepair recognition sites for three restriction enzymes. These larger 8 base-pair recognition sites occur less frequently throughout the yeast genome than the 6 base-pair sites present in the MCS of pAB1. Thus, the utilization of restriction enzymes that recognize 8 base-pair sequences to clone the various regulatory regions (engineered into the PCR primers used to amplify the regions) would minimize the occurrence of those sites within the regions themselves. To construct pAB2, pAB1 was linearized with XbaI and SphI, dropping out the existing MCS, and an adapter containing the new MCS was ligated in. The adapter was made by hybridizing two oligonucleotides, 8Cutter (5'-

CGGCGCGCCGCGGCCGCATGGCCGGCCAAT-3') and 8CutEnd (5'-CTAGATTGGCCGGCCATGCGGCCGCGGCGCGCCGCATG-3'). This adapter has sites for the restriction enzymes FseI, NotI, and AscI (underlined).

The promoter regions were cloned utilizing PCR of genomic DNA prepared from a strain derived from S288c; JRY147 (MATa SUC2 mal mel gal2 CUP1). The promoter-specific primers were designed such that the proximal primer spanned the start codon of the specific gene and included a few (usually four) codons derived from the gene. The position of the distal primer was determined on a case-by-case basis depending on the distance to, and orientation of, the neighboring open reading frame (ORF) and the restriction sites present. Where the upstream ORF was positioned in a divergent orientation and within 1,200 base-pairs, the size of the promoter fragment amplified was adjusted such that all nucleotides up to, but not including, the start codon of the upstream ORF were present. In cases where the upstream ORF was situated in the same orientation, the amplified fragment was designed to extend into the coding region but not so as to include the start codon. Both primers had restriction enzyme recognition sites engineered into the ends to allow the subsequent cloning of the PCR fragment into pAB1, or pAB2.

Construction of Reporter Gene Fusions (Method 2)

In another method for constructing genome reporter constructs, a vector comprising a marker gene having an amber mutation and a *supF* tRNA gene which suppresses the amber mutation is used as the parent vector.

A plasmid cloning vector was constructed which comprises a mutant β-lactamase gene with an amber mutation and a *supF* tRNA gene. Downstream of the *supF* tRNA gene there is a "stuffer" DNA fragment which is flanked by BsmBI restriction sites. The BsmBI restriction enzyme cuts outside of its six base pair recognition sequence (see, e.g., New England Biolabs 96/97 Catalog, p. 23) and creates a four nucleotide 5' overhang. When the plasmid cloning vector is digested with BsmBI, the enzyme cleaved within the stuffer DNA and within the adjoining tRNA gene and deleted the four 3' terminal nucleotides of the gene. The deleted *supF*

tRNA gene encodes a tRNA which cannot fold correctly and is non-functional, i.e., it could not suppress the amber mutation in the mutant β-lactamase gene (β-lactamase (amber)). Downstream from the stuffer DNA fragment is the coding region of a modified green fluorescent protein ("*GFP*") gene.

The stuffer DNA was excised from the vector by digestion with BsmBI. The double-stranded DNA at the *supF*-stuffer fragment junction, produced by BsmBI digestion, is shown below. The tRNA gene sequences are indicated in bold:

```
5' ..supF..TC          CCCCGGAGACGTC..stuffer..
          ..AGGGGG         CCTCTGCAG..5'
               BsmBI
```

The 3' terminal sequence of the *supF* gene necessary for proper function is TCCCCC**ACCA**. The vector, once cleaved with BsmBI, lacks the *supF* tRNA **ACCA** terminal nucleotides if the overhangs self-anneals during re-circularization of the plasmid in the absence of insert.

A DNA insert containing the upstream regulatory sequence from a yeast ORF was generated as a PCR fragment. Two oligonucleotides were designed to flank the DNA insert sequences of interest on a template DNA and anneal to opposite strands of the template DNA. These oligonucleotides also contained a sequence at their respective 5' ends that, when converted into a 5' overhang (in the double-stranded PCR fragment generated using the oligonucleotides), is complementary to the overhangs on the cloning vector generated by BsmBI endonucleolytic cleavage.

Oligonucleotide #1 comprises the 5' terminal sequence: 5' CCCC**ACCA** .... The remaining nucleotides 3' to this sequence were designed to anneal to sequences at one end of the DNA insert of choice, in this Example, to one of a multitude of yeast expression control sequences.

As highlighted in bold above, oligonucleotide #1 comprises the base pairs needed to restore the wild-type 3' terminal end of the *supF* tRNA gene. These base pairs are located immediately 3' to the sequence that allows the insert to anneal to the overhang in the BsmBI-digested pAB4 vector.

Oligonucleotide #2 comprises the 5' terminal sequence: 5' TCCTG .... The remaining nucleotides 3' to this sequence were designed to anneal to sequences at

the other end of the DNA insert of choice, in this Example, to one of a variety of yeast expression control sequences which may be used according to this invention.

The DNA template (*S. cerevisiae* genomic DNA) and the two oligonucleotides were annealed and the hybrids were amplified by polymerase chain reaction using Klentaq™ polymerase and PCR buffer according to the manufacturer's instructions (Clontech). Briefly, 15 ng *S. cerevisiae* genomic DNA served as template DNA in a 10µl PCR reaction containing 0.2mM dNTPs, PCR buffer, Klentaq™ polymerase, and 1 µL of an 8µM solution containing the primer pairs. The PCR reaction mixture was subjected to the following steps: a) 94°C for 3 min; b) 94°C for 15 sec; c) 52°C for 30 sec; d) 72°C for 1 min, 45 sec; and e) 4°C indefinitely. Steps b) through d) were repeated for a total of 30 cycles. The PCR amplification product was purified away from other components of the reaction by standard methods.

To generate the desired 5' overhangs on the ends of the PCR amplification product, the PCR fragment was treated with DNA polymerase I in the presence of dTTP and dCTP. Under these conditions, DNA polymerase I fills in 3' overhangs with its 5' to 3' polymerase activity and also generates 5' overhangs with its 3' to 5' exonucleolytic activity, which, in the presence of excess dTTP and dCTP, removes nucleotides in a 3' to 5' direction until a thymidine or a cytosine, respectively, is removed and then replaced.

The overhangs generated by this reaction are:

a) At the 5' end (*supF* tRNA restoring end) of the DNA insert:

```
5' CCCCACCA..        becomes      5' CCCCACCA..
       GGGGTGGT..                                TGGT..
```

b) At the 3' end of the DNA insert (joined to the *GFP* coding sequence):

```
5' CAGGA..       becomes     5' C
   GTCCT..                      GTCCT..
```

This DNA insert, now comprising 5' overhangs compatible with one of each of the ends of the BsmBI-cleaved pAB4 vector, was used as substrate in a standard ligation reaction with the BsmBI-cleaved pAB4 vector. The resulting ligation

mixture was used to transform competent *E. coli* cells. The cells were plated on agar plates in the presence of ampicillin.

Colonies that grew in the presence of ampicillin were producing functional β-lactamase enzyme and each harbored the desired recombinant DNA molecule, having a DNA insert with a yeast expression control sequence inserted upstream of the modified *GFP* coding region. The *supF* gene on vectors which re-ligated without a DNA insert did not express a functional *supF* tRNA and did not make functional β-lactamase. Thus, they were not found in transformed host cells grown on ampicillin.

Construction of Yeast Strains

Strain ABY11 (MATa leu2Δ1 ura3-52) of *S. cerevisiae* was used. ABY11 is derived from S288c. GRM arrays were grown at 30°C on solid casamino acid medium (Difco) with 2% glucose and 0.5% UltraPure Agarose (Gibco BRL). The medium was supplemented with additional amino acids and adenine (Sigma) at the following concentrations: adenine and tryptophan at 30 μg/ml; histidine, methionine, and tyrosine at 20 μg/ml; leucine and lysine at 40 μg/ml. Stock solutions of the supplements were made at 100x concentrations in water. Yeast cells were transformed with the reporter plasmids prepared by Method 1 or Method 2 (above) by the lithium acetate method (Ito et al., 1983, and Schiestl and Gietz, 1989).

Determinations of Reporter Gene Expression Levels

Solutions of test compounds were added directly to the yeast strains or were coated on plates prior to addition of the yeast strains. The individual strains comprising the GRM were maintained as independent colonies (and cultures) in a 96-well format, in medium selecting for the URA3-containing reporter plasmid. Prior to each experiment, fresh dilutions of the reporter-containing strains were inoculated and grown overnight at 30°C. A Hamilton MicroLab 4200, a multichannel gantry robot equipped with a custom pin tool device capable of dispensing 50 nanoliter volumes in a highly reproducible manner, was used to array the matrix of yeast strains in a uniform

manner onto solid agar growth media at a density of 1536 reporter strains per 110 cm^2 plate. Fifty nanoliters of yeast liquid cultures arrayed onto solid medium by the Hamilton MicroLab 4200 results in colony-to-colony signal reproducibility of less than 5% variation. Once arrayed, each plate was grown at 30°C for 18 hours or at 25°C for 24 hours.

The level of fluorescence expressed from each reporter gene fusion was determined using a Molecular Dynamics Fluorimager SI. AIS image analysis software (Imaging Research, Ontario CA) was used to quantitate the fluorescence of each colony in the images. Generally, the drug treatments were performed at several concentrations, with the analysis based upon the concentration producing the most informative expression profile.

EXAMPLE 2: IDENTIFICATION OF *HES1* AS A REGULON INDICATOR GENE

The effects of Simvastatin on the Genome Reporter Matrix™ were tested at a concentration of 20 µg/ml. The *HES1* reporter gene construct was induced by a natural log ratio of 4.2 (treated/untreated), indicating that the *HES1* reporter had an excellent signal to noise ratio induction in response to Simvastatin. The *HES1* gene encodes a protein with a significant amount of similarity with oxysterol binding proteins and has been implicated in isoprenoid metabolism (**Figure 35**). Analysis of gene expression data with the Genome Reporter Matrix™ revealed that *HES1* expression is highly correlated with expression of genes encoding enzymes of the isoprenoid biosynthetic pathway (**Figure 36**).

The specificity of the *HES1* reporter for inhibitors of ergosterol biosynthesis was tested *in silico*. The expression of the *HES1* reporter was examined in data from 710 experimental treatments of the Genome Reporter Matrix™. Basal levels of *HES1* reporter gene expression were 0.1 units. Units are defined as an arbitrary fluorescent value that has been normalized such that a value of 1.0 equals the mean reporter fluorescent level of all members of the Genome Reporter Matrix™ in a given experiment. All treatments (a total of 51) that induced *HES1* reporter gene

levels to 0.5 units or greater were treatments known to inhibit ergosterol biosynthesis, indicating a high degree of specificity for this pathway (**Figure 37**).

The utility of the *HES1* reporter gene in a high-throughput screen was tested by incubating a yeast strain harboring the *HES1* reporter in a 384-well array containing various concentraions of ergosterol biosynthesis inhibitors (Econazole and Simvastatin) and nonspecific drugs (Flucytosine and Nifedipine). Cells were grown to mid-log phase at 30°C in casamino acids medium (0.67% yeast nitrogen base, 2% glucose, 2% casamino acids). Cell density was adjusted prior to incubation in various concentrations of drug. Arrays were incubated at 30°C for 24 hrs prior to imaging. The *HES1* reporter was found to be specifically induced by Econazole and Simvastatin but not by Flucytosine or Nifedipine.

To further test the viability of this indicator gene in a high-throughput screen, the regulation of the *HES1* reporter was tested in two different strain backgrounds. ABY11 (*MATa leu2Δ1 ura3-52)* is a wild-type strain. ABY140 (*MATa his3Δ1 leu2Δ0 met15Δ0 pdr5::KanMX ura3Δ0 yor1::KanMX*) is a strain containing mutations in two multidrug resistance genes. Induction of the *HES1* reporter gene in ABY140 was found to be more sensitive to Simvastain and Econazole but not to Flucytosine or Nifedipine when compared to ABY11.

The ABY140 [*HES1*] strain was used to screen approximately 16,800 chemicals from a combinatorial chemistry library. One percent of these chemicals induced the *HES1* indicator gene. Twenty-four of these chemical were further tested in a secondary screen for the ability to induce four additional indicator (also referred to as reporter) genes whose expression are also coordinately regulated with genes encoding ergosterol biosynthetic enzymes. Eight of these twenty-four chemicals also induced these reporter genes, suggesting that these chemicals interfere with ergosterol biosynthesis.

This example reveals how a high quality promoter sequence identified from systematic genome expression data can be employed with a significant degree of confidence to identify chemicals with a desired biological activity.

The DNA and amino acid sequence of *HES1* is shown in **Figures 62 and 63**, respectively.

EXAMPLE 3: IDENTIFICATION OF *YJL105w* AS A TARGET GENE

YJL105w was a previously uncharacterized ORF which contains a PHD finger suggesting that it functions as a transcription factor **(Figure 1)**. Gene expression correlation coefficients were calculated for 1532 reporter constructs including known genes involved in sterol biosynthesis. Several uncharacterized genes, including *YJL105w*, were found to have highly correlated gene expression with genes encoding sterol biosynthetic enzymes. *YJL105w* expression correlated very well (0.83) with expression of *CYB5*, a gene involved in ergosterol biosynthesis **(Figure 2)**. Cyb5p is thought to be an electron donor for sterol modifying enzymes (Mitchell A.G., Martin C.E., *J. Biol. Chem.*, 1995, **270**(50):29766-72). Expression of *YJL105w* was induced considerably by drugs that inhibit sterol biosynthesis as well as by a mutation in the gene encoding HMG-CoA Synthase **(Figure 3)**. The *YJL105w* reporter construct comprises 1200 base-pairs of DNA sequence 5' to the ATG start codon and thus, contains sequence information sufficient to confer the observed regulated expression.

To test whether *YJL105w* has a role in isoprenoid metabolism, a *yjl105w* mutant where the entire ORF was replaced with the kanamycin resistance gene was constructed. Approximately 5 x 10^6 cells of the *yjl105w* mutant strain and a wild-type control strain (ABY363, MATα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*) were plated onto separate non-selective agar plates. The sterol biosynthetic inhibitor lovastatin (250μg) was applied to a sterile disk on each lawn and the cells were allowed to grow overnight at 30°C. The *yjl105w* mutant strain was found to be significantly more resistant to lovastatin treatment, further implicating this ORF in lipid metabolism **(Figure 4)**.

YJL105w appears to be fungal-specific since no apparent mammalian counterparts were found. Although *YJL105w* is not an essential gene, it could provide utility for constructing strains for specific applications. For instance, the resistance to

lovastatin conferred by a *yjl105w* mutant could result from an elevated flux through the isoprenoid biosynthetic pathway. Such a condition may result from an altered composition of the cell's lipid bilayer that triggers the induction of synthesis of isoprenoid biosynthetic enzymes and/or reduces the cell's permeability to lovastatin. In either of these cases, a strain defective for *YJL105w* could be useful for constructing strains that could grow under extreme situations, such as in industrial applications. Examples of extreme conditions include growth at high or low temperatures (>35°C or <20°C) or in osmotically stressful conditions or in the presence of amphipathic solutes. Alternatively, the resistance to lovastatin in the *yjl105w* mutant could result from decreased expression of membrane transporters or channels that allow entry of foreign compounds (xenobiotics). In this case, overexpression of *YJL105w* could produce a highly permeablized strain that would have numerous applications where entry of compounds into a cell is limited by permeability or availability of compounds. A mammalian counterpart of this ORF, if found, could be useful as a diagnostic marker for people with high serum cholesterol levels. Individuals that have mutations, null or weak (hypomorphic) alleles, might be expected to have a higher rate of sterol synthesis.

The DNA and protein sequences of *YJL105w* are depicted in Figures 39 and 40, respectively.

EXAMPLE 4: IDENTIFICATION OF *YMR134w* AS A TARGET GENE

YMR134w is an ORF that had been suggested previously to be involved in iron metabolism (**Figure 5**). Among 1532 reporter constructs, *YMR134w* expression was found to be highly correlated with the expression of *ERG2* (**Figure 6**) and is therefore likely to be involved in lipid metabolism. The *YMR134w* reporter construct was found to be highly induced by various statins (inhibitors of HMG-CoA reductase) and azole compounds (inhibitors of lanosterol 14-alpha demethylase, *ERG11*) (**Figure 7**). The *YMR134w* reporter construct comprises 1200 base-pairs of DNA sequence 5' to the ATG start codon and thus, contains sequence information sufficient to confer the observed regulated expression. A database search for

YMR134w-related protein sequences revealed a weak similarity to human vascular endothelial growth factor receptor (**Figure 8**).

The DNA and protein sequences of *YMR134w* are depicted in **Figures 41 and 42**, respectively.

EXAMPLE 5: IDENTIFICATION OF *YER044c* AS A TARGET GENE

YER044c was a previously uncharacterized yeast ORF with one predicted transmembrane domain (**Figure 9**). *YER044c* expression is significantly correlated with the expression of *ERG2* (0.82, **Figure 10**). Statins, azoles and a deletion mutant of the *ERG11* gene each induce expression of the *YER044c* reporter construct most significantly in 498 treatments of the GRM (**Figure 11**). The *YER044c* reporter construct comprises 1200 base-pairs of DNA sequence 5' to the ATG start codon and thus contains sequence information sufficient to confer the observed regulated expression. DNA and proteins sequence database comparisons with the predicted protein sequence of *YER044c* revealed an apparent *Schizosaccharomyces pombe* counterpart and numerous mammalian EST apparent counterparts (**Figures 12-14**).

The DNA and protein sequences of *YER044c* are depicted in **Figures 43 and 44** respectively. The apparent mouse, human and rat EST counterparts of *YER044c* are depicted in **Figures 45-47**, respectively.

EXAMPLE 6: IDENTIFICATION OF *YLR100w* AS A TARGET GENE

YLR100w was a previously uncharacterized yeast ORF (**Figure 15**). Expression of *YLR100w* correlated significantly (0.82) with *CYB5* in the GRM composed of 6036 reporter constructs in 706 experimental treatments. The correlation of expression of *YLR100w* to the expression of *CYB5* implied a role of *YLR100w* in lipid metabolism. Expression of the *YLR100w* reporter was induced significantly by statins, azoles and in a yeast *erg11* mutant consistent with a role of *YLR100w* in lipid metabolism (**Figure 17**). Searches of DNA and protein sequence databases for similar

sequences revealed a GenBank entry for a 17-beta-hydroxysteroid dehydrogenase mouse cDNA (**Figure 18**).

The sequence of the mouse cDNA is shown in **Figure 53**. Given the protein sequence similarity (**Figure 19**) and the fact that yeast is not known to synthesize steroid hormones, it is conceivable that the mouse cDNA encodes a protein with another role in lipid metabolism. In this case, the mammalian protein could have utility as a pharmacological target to modulate lipid metabolism. Another GenBank entry was found for a rat ovarian specific protein with significant similarity to *YLR100w*. The sequence of the rat protein is shown in **Figure 65**. Two mouse ESTs were found to be significantly similar to *YLR100w*. The sequence of the two mouse ESTs are shown in **Figures 51 and 52**. A human EST was found that was similar to *YLR100w*, but to a lesser extent than the two mouse ESTs.

The DNA and protein sequences of *YLR100w* are depicted in **Figures 48 and 49**, respectively. The sequence of the human EST is shown in **Figure 50**.

EXAMPLE 7: IDENTIFICATION OF *YER034w* AS A TARGET GENE

YER034w is a yeast ORF that had been shown previously not to be essential for cell viability (**Figure 20**). Expression of the *YER034w* reporter construct was found to be correlated (0.75) with the expression of a *GPA2* reporter construct in a GRM composed of 1532 reporters treated under 498 experimental conditions (**Figure 21**). *GPA2* encodes the alpha subunit of a trimeric G protein involved in pseudohyphal differentiation (Lorentz, M.C. and Heitman, J. *EMBO J.* 1997 **16**:7008-7018). This correlation suggested that *YER034w* had a role in the pseudohyphal growth and could represent a new antifungal target.

To test this hypothesis, a diploid homozygous *yer034w* knockout strain was purchased from Research Genetics (Huntsville, AL). Wild-type cells (ABY13, *MATa/MATalpha his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 met15Δ0/MET15 LYS2/lys2Δ0 ura3Δ0/ura3Δ0*) and the homozygous *yer034w* knockout strain were plated onto low nitrogen plates to stimulate pseudohyphal differentiation. After four days at 25°C, plates were examined under a microscope. The *yer034w* knockout strain had

undergone significantly more differentiation than the wild-type control both in terms of numbers of projections per colony (**Figure 22**) and the size of the hyphae. This result implicated *YER034w* in the dimorphic transition of cells from yeast to pseudohyphae. The ability of fungi to undergo this morphological transition has been suggested to be a critical aspect of fungal pathogenicity. A search for related mammalian protein sequences did not identify any obvious counterparts suggesting that this protein is fungal-specific and may be an amenable anti-fungal target.

The DNA and protein sequences of YER034w are depicted in **Figures 54 and 55**, respectively.

EXAMPLE 8: IDENTIFICATION OF *YKL0774w* AS A TARGET GENE

YKL077w was a previously uncharacterized ORF with one predicted transmembrane domain (**Figure 23**). Expression of the *YKL077w* reporter construct was found to be correlated (0.92) with the expression of a *SGV1* reporter construct in a GRM composed of 1532 reporters treated under 498 experimental conditions (**Figure 24**). Sgv1p is a Cdc28p-related protein kinase that is essential for cell viability. In addition to Sgv1p expression, *YKL077w* expression correlated highly (>0.8) with *PKC1* and *RHO1* (**Figure 25**), genes involved in cell wall integrity and cytoskeletal reorganization. Database searches with the predicted protein sequence of *YKL077w* did not identify apparent mammalian counterparts (**Figure 26**). *YKL077w* could represent an antifungal target given the lack of a mammalian homolog and its proposed involvement in cellular structure and/or proliferation. Nevertheless, in the event a mammalian counterpart is discovered, it could represent an anti-proliferative target as well.

The DNA and protein sequences of *YKL077w* are depicted in **Figures 56 and 57**, respectively.

EXAMPLE 9: IDENTIFICATION OF *YGR046w* AS A TARGET GENE

YGR046w was a previously uncharacterized yeast ORF that has been shown to be essential for viability (**Figure 27**). Expression of *YGR046w* correlated

significantly (0.90) with *IRA2* in the GRM composed of 6036 reporter constructs in 706 experimental treatments (**Figure 28**). Ira2p is a GTPase activating protein (GAP) for Ras1p and Ras2p. In addition to *IRA2* expression, *YGR046w* expression correlated very well (>0.77) with the expression of known genes involved cell proliferation functions (**Figure 29**). The expression of *YGR046w* was found to be most sensitive to agents that disrupt mitochondrial function, create oxidative stress and disrupt the cytoskeleton (**Figure 30**).

Given its proposed involvement in cell proliferation, *YGR046w* could represent a target for modulation of cell growth. A search of protein and DNA sequence databases did not reveal any apparent mammalian homologs. Nevertheless, if such a sequence is identified, it may represent an anti-proliferative mammalian target.

The DNA and protein sequences of *YGR046w* are depicted in **Figures 58 and 59**, respectively.

EXAMPLE 10: IDENTIFICATION OF *YJR041c* AS A TARGET GENE

Mutant strains defective for *YJR041c* have been shown previously to display a severe growth defect, but no function for *YJR041c* was known (**Figure 31**). Expression of *YJR041c* correlated significantly (0.83) with *MED7* in the GRM composed of 6036 reporter constructs in 706 experimental treatments (**Figure 32**). Med7p encodes a component of the mediator complex involved in RNA polymerase II transcription. *YJR041c* expression was also found to correlate significantly (>0.71) with several genes involved in different aspects of RNA metabolism. These processes include RNA polymerase I and II transcription, mRNA splicing, RNA turnover and ribosome function (**Figure 33**).

Database searches for related sequence identified similar sequences from *Schizosaccharomyces pombe* (**Figure 34**). No obvious mammalian counterparts were identified suggesting that *YJR041c* is a fungal-specific protein. Given these factors, *YJR041c* could represent an attractive target for antifungal therapy. In the event a mammalian counterpart is identified, it also could represent a target with utility for modulating cell proliferation.

The DNA and protein sequences of *YJR041c* are shown in **Figures 60 and 61**, respectively.

EXAMPLE 11: SCREENING ASSAY USING THE GENOME REPORTER MATRIX™ TO IDENTIFY TARGET INHIBITORS

A mutant or conditional allele of target yeast gene is produced as discussed above. The allele may be conditional either for function or expression. For instance, the conditional allele may be a temperature-sensitive allele of the target gene or the target gene may be operably linked to an inducible promoter for regulated expression. In a preferred embodiment, the target gene is operably linked to an inducible promoter that permits expression anywhere between 0% and 500% of wild type expression. The target gene of interest is transfected and expressed in yeast cells of the GRM that have a functional deletion of the target gene of interest. The level of expression of the conditional allele is varied between 0% and 500% of wild type expression, and the expression of the reporter constructs of the GRM is measured in response to the expression of the target gene. The expression of the reporter constructs is then correlated to the expression of the target gene. Thus, one can identify a subset of genes that are either induced or repressed by overexpression of the target gene.

The yeast strains containing the subset of genes whose expression is dependent upon overexpression, and thus the function of the essential gene, are then used to screen compounds that are potential target inhibitors. The yeast strains are incubated with the compounds. If a reporter gene in a particular yeast strain is induced by overexpression of the target gene, then potential inhibitors are screened for the ability to downregulate the reporter gene. Conversely, if a reporter gene is repressed by overexpression of the target gene, then potential inhibitors are screened for the ability to upregulate the reporter gene. Potential inhibitors are screened for the ability to appropriately upregulate and downregulate a number of the genes whose expression is dependent upon expression or overexpression of the target gene. When potential target inhibitors are identified, these candidate compounds are tested for their ability to

inhibit the pathway that the target gene is part of. For instance, if the target gene is *YER034w*, then the inhibitor may be tested for antifungal activity.

If a target gene has a plant or animal counterpart, one may express the plant or animal counterpart in a yeast strain lacking the target gene to see if the plant or animal counterpart can functionally substitute for the yeast gene. If it can, then the plant or animal counterpart can be used in the above example to screen for potential targets for either a plant or animal inhibitor. This is especially useful if the target gene has a mammalian counterpart. Similarly, even if a plant, animal or mammalian counterpart has not been identified, potential inhibitors may be tested for their ability to inhibit the pathway that the target gene is part of, if that pathway is shared by yeast and higher eukaryotes.

EXAMPLE 12: SIMULTANEOUS TRACKING OF MULTIPLE REPORTERS AS REGULON INDICATOR GENES

The effects of inactivating an osmotic stress pathway were tested by deleting a pathway component (Hog1p stress-activated protein kinase). Using the *hog1* knock-out profile as model, multiple RIGs that would specifically indicate pathway inhibitors were identified and tested *in silico* by examining all conditions in which selected RIGs were activated or repressed. It was determined that simultaneously monitoring up-regulation of *PGU1* and down-regulation of *DAK1* gave good specificity for pathway inactivation as determined by the separation of the hog1 knock-out profile from all other conditions in which these two reporters were affected (**Figure 74**). In this example, RIGs were not part of the target regulon but were chosen empirically based on behavior under all conditions.

Similarly, 2 RIGs were identified that could specifically indicate mitochondrial inactivation by comparing the behavior these RIGs in the subset of treatments that target mitochondria with all treatments that affect these RIGs. It was determined that simultaneously measuring up-regulation of 2 RIGs (*STE18* and *YGL198w*) provides good specificity for mitochondrial perturbations as determined by

the separation of this subset of common treatments from all other conditions that affect these RIGs (**Figure 75**).

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

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CLAIMS

We claim:

1. A method for placing Gene X, a gene of unknown function, into a functional genetic group comprising the steps of:

a) generating a gene expression profile for Gene X;

b) comparing the gene expression profile of Gene X with gene expression profiles of a plurality of other genes in a database of compiled gene expression profiles to generate expression correlation coefficients;

c) identifying based on their expression correlation coefficients a set of genes comprising Gene X that are coordinately expressed;

d) determining if the one or more genes whose expression is most highly correlated with that of Gene X belong to a gene regulon involved in a known biological pathway, or a common set of biological reactions or functions; and

e) optionally testing the effect on Gene X expression of at least one altered condition or treatment known to affect the function to which Gene X hs been ascribed;

wherein Gene X is placed in the gene regulon of d) if Gene X expression is coordinate with expression of that regulon.

2. A method for identifying a regulon indicator gene in a database of compiled gene expression profiles, wherein expression of the regulon indicator gene correlates with the expression of at least one known gene in a group of coordinately expressed genes or provide a measure of the function of a biological process of interest, the method comprising the steps of:

a) comparing gene expression profiles of a plurality of genes in the database to generate expression correlation coefficients;

b) identifying based on their relative expression correlation coefficients a set of genes that are coordinately expressed;

c) selecting a set of genes from b) which comprises one or more genes known to function in a particular biological pathway, or a common set of biological reactions or functions;

d) selecting a member of the set of c) having one or more of the following characteristics:

1) its expression profile is sensitive to one or more stimuli;

2) its expression profile exhibits a large dynamic range in response to one or more stimuli;

3) its expression profile exhibits a rapid kinetic response to one or more stimuli;

4) its expression profile is specific to a known biological pathway or a common set of biological reactions or functions;

5) the regulon indicator gene does not contain sequences that are problematic for maintaining on plasmids when introduced into host cells.

3. The method of claim 2, wherein the regulon indicator gene is co-regulated with one or more genes in the group of coordinately expressed genes of c).

4. The method of claim 2, wherein the regulon indicator gene, upon expression, controls the expression of at least one other gene in the group of coordinately expressed genes of c).

5. The method of claim 2, wherein the regulon indicator gene is of previously unknown function.

6. A method for selecting a novel regulon target gene from a database of compiled gene expression profiles, comprising the steps of:

a) comparing gene expression profiles of a plurality of genes in the database to generate expression correlation coefficients;

b) identifying based on their expression correlation coefficients a set of genes that are coordinately expressed;

c) selecting from b) a set of genes comprising one or more genes of unknown function and one or more genes known to function in a particular biological pathway, or a common set of biological reactions or functions of interest;

d) selecting from the set of c) at least one gene of unknown function, Gene X, as a novel regulon target gene; wherein Gene X is a gene whose expression profile closely correlates to the expression profiles of the one or more genes of the set of c) known to function in the particular biological pathway, or common set of biological reactions or functions of interest.

7. The method of claim 6, further comprising the step of generating individual correlation coefficients between the gene expression profile of Gene X and a plurality of genes in the database to assess the selectivity of Gene X as a novel regulon target gene.

8. The method of claim 6, further comprising the step of determining whether the protein encoded by Gene X exhibits substantial homology to a human, non-human mammal, avian, amphibian, fish, insect or plant protein.

9. The method of claim 8, wherein said determining comprises the steps of hybridizing Gene X to genomic DNA from human, non-human mammal, avian, amphibian, fish, insect or plant cells or tissue under low stringency conditions.

10. The method of claim 8, wherein said determining comprises the steps of:

a) comparing the DNA sequence of Gene X to the DNA sequences from other organisms or

b) obtaining an amino acid sequence encoded by Gene X and comparing it to amino acid sequences from other organisms.

11. The method of any one of claims 8-10, wherein the DNA or amino acid sequences from other organisms are contained within a database, and wherein the DNA or amino acid sequence encoded by Gene X is compared to the DNA or amino acid sequences from other organisms using a computer algorithm.

12. The method of claim 11, wherein the computer algorithm is blastp, tblastn or another algorithm that utilizes string alignments.

13. The method of claim 6, further comprising the steps of:

a) disrupting the function of Gene X or its homolog in a yeast cell; and

b) identifying whether the function of Gene X is essential for yeast germination, vegetative growth, pseudohyphal or hyphal growth.

14. A method for identifying a potential inhibitor of a regulon target gene, comprising the steps of:

a) incubating a polypeptide comprising an amino acid sequence encoded by a regulon target gene with a compound under conditions effective to promote specific binding between the polypeptide and the compound; and

b) determining whether the polypeptide bound to the compound;

wherein the compound is a potential inhibitor if the compound binds to the polypeptide.

15. The method of claim 14, wherein the polypeptide comprises the full-length amino acid sequence encoded by the regulon target gene.

16. The method of claim 14, wherein the polypeptide comprises a functional fragment of the amino acid sequence encoded by the regulon target gene.

17. The method of claim 14, wherein the polypeptide is a fusion protein comprising an epitope tag or reporter gene.

18. The method of claim 14, wherein the polypeptide is attached to a solid support surface and the compound is in mobile phase.

19. The method of claim 14, wherein the compound is attached to a solid support surface and the polypeptide is in mobile phase.

20. The method of claim 14, wherein the compound is a library selected from the group consisting of a combinatorial small organic library, a phage display library and a combinatorial peptide library.

21. The method of claim 14, wherein said determining is performed by ELISA, RIA or BiaCORE analysis.

22. The method of claim 14, wherein said determining is performed by high throughput screening.

23. The method of claim 14, further comprising the step, performed before step a), of expressing in a host cell a regulon target gene.

24. The method of claim 14, wherein the target gene is selected from the group consisting of *YMR134w*, *YER034w*, *YJL105w*, *YKL077w*, *YGR046w*, *YJR041c*, *YER044c* and *YLR100w* and their mammalian homologs.

25. The method of claim 14, wherein the target gene is human EST W28235, a homolog of *YER044c*.

26. The method of claim 14, wherein the target gene is human EST R92053, a homolog of *YLR100w*.

27. The method of claim 14, wherein the target gene is mouse EST AI386195, a homolog of *YER044c*.

28. The method of claim 14, wherein the target gene is mouse EST AI226514, a homolog of *YLR100w*.

29. The method of claim 14, wherein the target gene is mouse EST AI528381, a homolog of *YLR100w*.

30. The method of claim 14, wherein the target gene is mouse gene 3319971, a homolog of *YLR100w*.

31. The method of claim 14, wherein the target gene is rat gene 1397235, a homolog of *YLR100w*.

32. The method of claim 14, further comprising performing, before step a), the step of expressing in a host cell a regulon target gene selected from the group consisting of *YMR134w*, *YER034w*, *YJL105w*, *YKL077w*, *YGR046w*, *YJR041c*, *YER044c* and *YLR100w* and their mammalian homologs.

33. A method for identifying a potential inhibitor of a regulon target gene, comprising the steps of:

a) creating a host cell in which the target gene has been altered or inactivated by mutation;

b) comparing gene expression profiles in the mutated host cell to those in a host cell which expresses the normal target gene;

c) identifying one or more potential target-dependent reporter genes whose expression is altered in the host cell in which the target gene has been altered or inactivated compared to the host cell which expresses the normal target gene;

d) screening one or more compounds for their effects on expression of the target-dependent reporter gene;

wherein if expression of the target-dependent reporter gene increases in the host cell harboring an altered or inactivated target gene, then a potential inhibitor of the regulon target gene will increase expression of the target-dependent reporter gene, and if expression of the target-dependent reporter gene decreases in the host cell harboring an altered or inactivated target gene, then a potential inhibitor of the regulon target gene will decrease expression of the target-dependent reporter gene.

34. The method of claim 33, further comprising the step, performed before step d), of assessing the specificity of a potential target-dependent reporter gene by comparing gene expression profiles the potential target-dependent reporter gene to a plurality of genes in a database of compiled gene expression profiles to generate individual expression correlation coefficients wherein a target-dependent reporter gene whose expression correlates with the expression of the regulon target gene and with a minimal number or no other gene is selected over one whose expression correlates with a greater number of genes based on expression correlation coefficients.

35. The method of claim 33 or 34, wherein upstream sequences that control expression of the target-dependent reporter gene are fused to a heterologous

coding sequence and that fusion used to screen compounds for potential inhibitors of the regulon target gene.

36. The method of claim 35, wherein the heterologous sequence comprises an epitope tag or a reporter gene.

37. The method of claim 35, wherein the fusion polypeptide is attached to a solid support surface and the compound is in mobile phase.

38. The method of claim 35, wherein the compound is attached to a solid support surface and the fusion polypeptide is in mobile phase.

39. The method of claim 33, wherein the compound is a library selected from the group consisting of a combinatorial small organic library, a phage display library and a combinatorial peptide library.

40. The method of claim 33, wherein said screening is performed by ELISA, RIA or BiaCORE analysis.

41. The method of claim 33, wherein said screening is performed by high throughput screening.

42. The method of claim 33, wherein the target gene is selected from the group consisting of *YMR134w*, *YER034w*, *YJL105w*, *YKL077w*, *YGR046w*, *YJR041c*, *YER044c* and *YLR100w* and their mammalian homologs.

44. The method of claim 33, wherein the target gene is human EST W28235, a homolog of *YER044c*.

45. The method of claim 33, wherein the target gene is human EST R92053, a homolog of *YLR100w*.

46. The method of claim 33, wherein the target gene is mouse EST AI386195, a homolog of *YER044c*.

47. The method of claim 33, wherein the target gene is mouse EST AI226514, a homolog of *YLR100w*.

48. The method of claim 33, wherein the target gene is mouse EST AI528381, a homolog of *YLR100w*.

49. The method of claim 33, wherein the target gene is mouse gene 3319971, a homolog of *YLR100w*.

50. The method of claim 33, wherein the target gene is rat gene 1397235, a homolog of *YLR100w*..

51. A method for inhibiting the expression of a regulon target gene in a host cell comprising the step of introducing into the host cell an inhibitor made according to any one of claims

52. The method of claim 51, wherein the target gene is selected from the group consisting of *YMR134w*, *YER034w*, *YJL105w*, *YKL077w*, *YGR046w*, *YJR041c*, *YER044c* and *YLR100w* and their mammalian homologs.

53. An antisense oligonucleotide comprising a sequence complementary to the sequence of an mRNA of a regulon target gene and effective to decrease transcription or translation of the gene.

54. The antisense oligonucleotide of claim 53 complementary to the sequence of the mRNA of a target gene selected from the group consisting of *YMR134w*, *YER034w*, *YJL105w*, *YKL077w*, *YGR046w*, *YJR041c*, *YER044c* and *YLR100w* and their mammalian homologs.

55. A ribozyme comprising a sequence complementary to the sequence of an mRNA of a regulon target gene and effective to decrease transcription or translation of the gene.

56. The ribozyme of claim 55 complementary to the sequence of the mRNA of a target gene selected from the group consisting of *YMR134w*, *YER034w*, *YJL105w*, *YKL077w*, *YGR046w*, *YJR041c*, *YER044c* and *YLR100w* and their mammalian homologs.

57. A neutralizing antibody to a protein encoded by a regulon target gene of a yeast or its mammalian homolog.

58. The neutralizing antibody of claim 57, wherein the target gene is selected from the group consisting of *YMR134w*, *YER034w*, *YJL105w*, *YKL077w*, *YGR046w*, *YJR041c*, *YER044c* and *YLR100w* and their mammalian homologs.

59. A fusion protein comprising an amino acid sequence encoded by a regulon target gene of a yeast or its mammalian homolog and further comprising an epitope tag or a reporter gene.

60. The fusion protein of claim 59, wherein the target gene is selected from the group consisting of *YMR134w*, *YER034w*, *YJL105w*, *YKL077w*, *YGR046w*, *YJR041c*, *YER044c* and *YLR100w* and their mammalian homologs.

61. A method for identifying a gene regulated by a regulon target gene of a yeast or its mammalian homolog, comprising the steps of:

a) overexpressing the target gene in host cells of a matrix comprising a plurality of units of cells, the cells in each unit containing a reporter gene operably linked to an expression control sequence derived from a gene of a selected organism; and

b) identifying genes that are either induced or repressed by overexpression of the target gene.

62. The method according to claim 61, wherein the target gene is selected from the group consisting of *YMR134w*, *YER034w*, *YJL105w*, *YKL077w*, *YGR046w*, *YJR041c*, *YER044c* and *YLR100w* and their mammalian homologs.

63. A method for identifying a regulon indicator gene in a database of compiled gene expression profiles, wherein expression of the regulon indicator gene provides a measure of the function of a biological pathway or process of interest, the method comprising the steps of:

a) examining exemplary expression profiles in response to one or more chemical or genetic treatments which target the pathway or process of interest to generate reporter sensitivity data;

b) selecting a set of genes from a) which comprises one or more genes most significantly affected in response to the treatment or treatments; and

c) selecting at least one gene from b) whose expression profile is maximized for its specificity and sensitivity to the treatment or class of treatments in a) compared to its sensitivity to all other treatments in the database.

64. The method of claim 63, wherein the regulon indicator gene is co-regulated with one or more genes in the set of genes of a).

65. The method of claim 63, wherein the regulon indicator gene, upon expression, controls the expression of at least one other gene in the set of genes of a).

YJL105w

GenBank No.	1008286
Chromosome	X
Protein	559 amino acids
	63,867 Daltons
Comments:	contains a PHD finger

Figure 1.

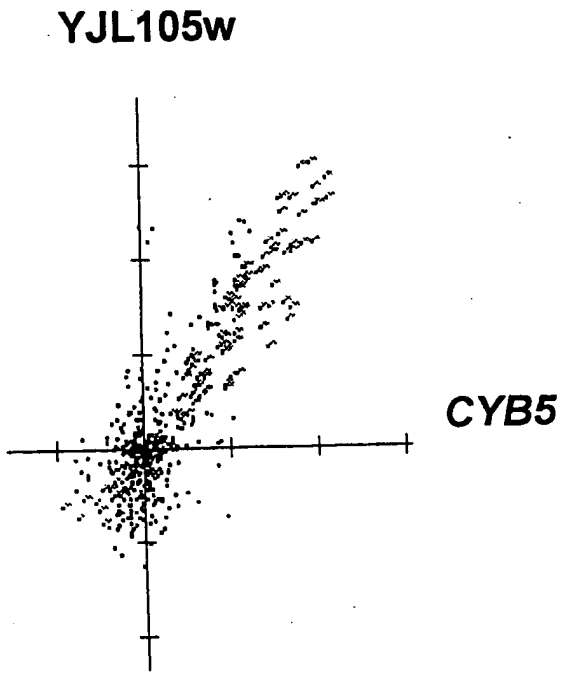


Figure 2.

Regulated Expression of YJL105w

Expt	Level	Natural Log Ratio	Treatment [baseline]
1455	9.1	+3.2	4.0ug/ml Fluvastatin - 18 hr [0.09]
1454	8.1	+3.1	8.0ug/ml Fluvastatin - 18 hr [0.13]
1537	7.9	+3.1	20ug/ml Lovastatin in 1 Ethanol - 18 hr [0.10]
1420	7.8	+3.1	20ug/ml Atorvastatin in 1 DMSO - 18 hr [0.14]
3455	7.8	+3.1	20ug/ml Lovastatin - 18 hr [0.20]
3456	7.8	+3.1	25ug/ml Lovastatin - 18 hr [0.20]
1944	6.5	+2.9	30ug/ml Mevastatin in 1.5 Ethanol - 18 hr [0.20]
1943	6.4	+2.9	15ug/ml Simvastatin in 1.5 Ethanol - 18 hr [0.13]
1554	5.8	+2.8	5ug/ml Simvastatin in 1 Ethanol - 18 hr [0.12]
1419	5.2	+2.7	30ug/ml Atorvastatin in 1 DMSO - 18 hr [0.12]
1553	5.1	+2.6	10ug/ml Simvastatin in 1 Ethanol - 18 hr [0.11]
3454	5.1	+2.6	10ug/ml Lovastatin - 18 hr [0.15]
1538	4.8	+2.6	10ug/ml Lovastatin in 1 Ethanol - 18 hr [0.09]
1421	4.4	+2.5	10ug/ml Atorvastatin in 1 DMSO - 18 hr [0.12]
1541	4.2	+2.4	10ug/ml Mevastatin in 1 Ethanol - 18 hr [0.08]
1456	4.1	+2.4	2.0ug/ml Fluvastatin - 18 hr [0.06]
1539	4.0	+2.4	5ug/ml Lovastatin in 1 Ethanol - 18 hr [0.08]
1540	4.0	+2.4	20ug/ml Mevastatin in 1 Ethanol - 18 hr [0.10]
2756	3.9	+2.4	[hmgs - ABY244.1 regulated (60)] - 18 hr [0.21]
2757	3.8	+2.3	[hmgs - ABY244.1 regulated (80)] - 18 hr [0.20]
2061	3.3	+2.2	35ug/ml Atorvastatin in 1 Ethanol - 18 hr [0.08]
1982	3.0	+2.1	0.125ug/ml Clotrimazole in 1 Methanol - 18 hr [0.19]
2060	2.9	+2.1	25ug/ml Atorvastatin in 1 Ethanol - 18 hr [0.07]
1542	2.8	+2.0	5ug/ml Mevastatin in 1 Ethanol - 18 hr [0.08]
1999	2.7	+2.0	20ug/ml Atorvastatin in 1 Ethanol - 18 hr [0.08]
3279	2.7	+2.0	0.15ug/ml Clotrimazole in 1 DMSO - 18 hr [0.13]
1935	2.6	+2.0	0.04ug/ml Econazole in 1 Methanol - 18 hr [0.18]
1478	2.5	+1.9	2.0ug/ml Fluconazole in 0.9 Saline - 18 hr [0.27]
1477	2.5	+1.9	3.0ug/ml Fluconazole in 0.9 Saline - 18 hr [0.31]
1983	2.5	+1.9	0.15ug/ml Clotrimazole in 1 Methanol - 18 hr [0.15]
3468	2.5	+1.9	20ug/ml Lovastatin [ABY139] - 18 hr [0.58]
2754	2.5	+1.9	[hmgs - ABY244.1 regulated (20)] - 18 hr [0.19]

Figure 3.

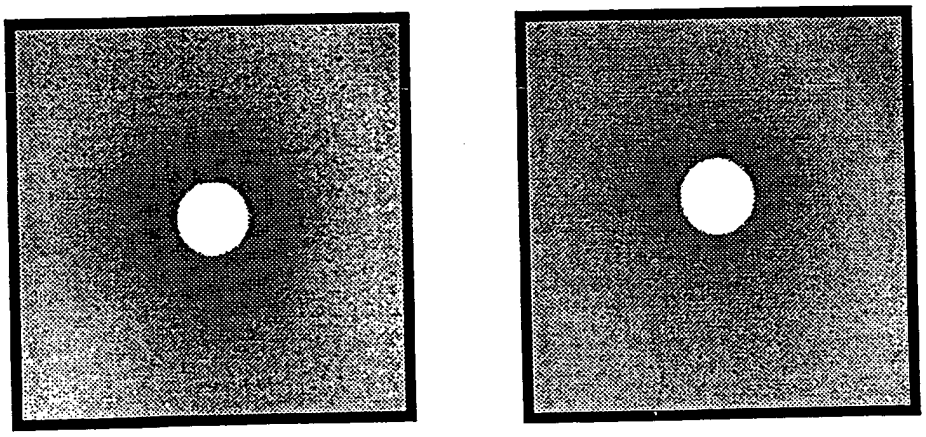


Figure 4.

YMR134w

GenBank No.	606432
Chromosome	XIII
Protein	236 amino acids
	27,911 Daltons
Comments:	involved in iron metabolism; potential transmembrane domain

Figure 5.

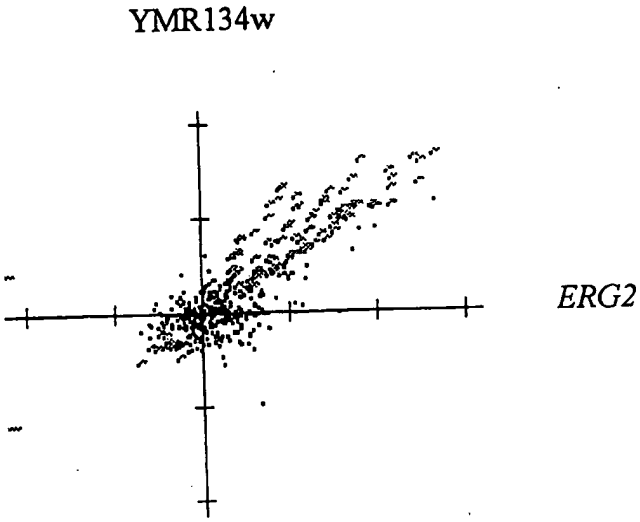


Figure 6.

Treatments Causing Highest Expression of YMR134w

Experiment	Level	log ratio	Treatment [baseline]
1943	1.3	+1.8	15ug/ml Simvastatin in 1.5 Ethanol - 18 hr [0.13]
1944	1.2	+1.7	30ug/ml Mevastatin in 1.5 Ethanol - 18 hr [0.20]
1419	1.2	+1.7	30ug/ml Atorvastatin in 1 DMSO - 18 hr [0.12]
1537	1.2	+1.7	20ug/ml Lovastatin in 1 Ethanol - 18 hr [0.10]
1454	1.2	+1.7	8.0ug/ml Fluvastatin - 18 hr [0.13]
1477	1.0	+1.5	3.0ug/ml Fluconazole in 0.9 Saline - 18 hr [0.31]
1553	0.9	+1.5	10ug/ml Simvastatin in 1 Ethanol - 18 hr [0.11]
1455	0.9	+1.5	4.0ug/ml Fluvastatin - 18 hr [0.09]
3455	0.9	+1.5	20ug/ml Lovastatin - 18 hr [0.20]
3456	0.9	+1.5	25ug/ml Lovastatin - 18 hr [0.20]
1538	0.9	+1.4	10ug/ml Lovastatin in 1 Ethanol - 18 hr [0.09]
3454	0.9	+1.4	10ug/ml Lovastatin - 18 hr [0.15]
1478	0.8	+1.4	2.0ug/ml Fluconazole in 0.9 Saline - 18 hr [0.27]
1540	0.8	+1.3	20ug/ml Mevastatin in 1 Ethanol - 18 hr [0.10]
1420	0.8	+1.3	20ug/ml Atorvastatin in 1 DMSO - 18 hr [0.14]
1611	0.8	+1.3	10ug/ml Fluconazole - 21 hr [0.04]
1554	0.7	+1.2	5ug/ml Simvastatin in 1 Ethanol - 18 hr [0.12]
3279	0.7	+1.2	0.15ug/ml Clotrimazole in 1 DMSO - 18 hr [0.13]
3469	0.7	+1.2	25ug/ml Lovastatin [ABY139] - 18 hr [0.57]
1605	0.7	+1.2	5ug/ml Fluconazole - 21 hr [0.04]
1936	0.7	+1.1	0.05ug/ml Econazole in 1 Methanol - 18 hr [0.14]
3468	0.7	+1.1	20ug/ml Lovastatin [ABY139] - 18 hr [0.58]

Figure 7.

Blastp search of GenBank

Sequences producing significant alignments:	Score (bits)	E Value
sp|P40207|YM17_YEAST HYPOTHETICAL 27.9 KD PROTEIN IN REC114-PSO...	483	e-136
sp|P17948|VGR1_HUMAN VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTO...	34	1.3
gi|3132831 (AF063657) vascular endothelial growth factor recept...	34	1.3
gi|2088746 (AF003142) contains similarity to C2H2-type zinc fin...	33	2.2
gi|886766 (U27832) Smt4p [Saccharomyces cerevisiae]	32	2.9
sp|P40537|SMT4_YEAST SMT4 PROTEIN >gi|1077779|pir||S49947 SMT4 ...	32	2.9
sp|P48034|ADO_BOVIN ALDEHYDE OXIDASE >gi|1149575|emb|CAA60701| ...	32	2.9
sp|Q06278|ADO_HUMAN ALDEHYDE OXIDASE >gi|2117502|pir||A49634 al...	32	5.0

.tblastn search of dbest

No hits found

Figure 8.

YER044c

GenBank No.	603277
Chromosome	V
Protein	148 amino acids
	17,140 Daltons
Comments:	unknown function; potential transmembrane domain

Figure 9.

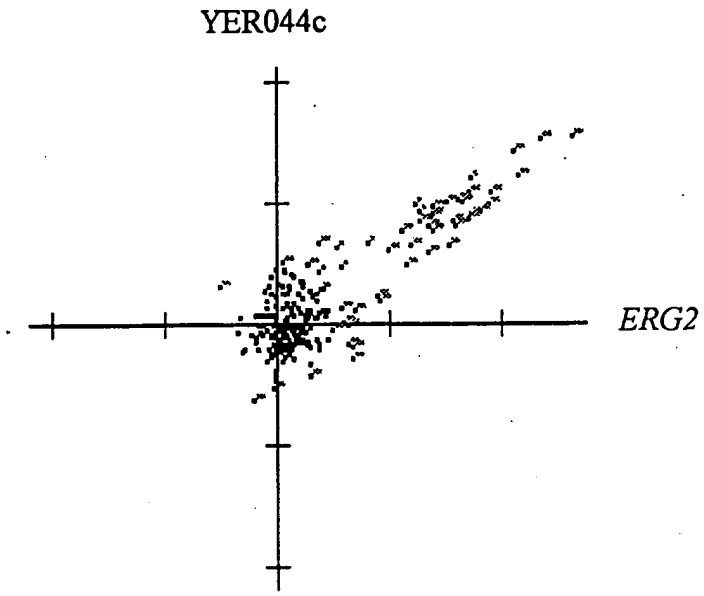


Figure 10.

Treatments Causing Highest Expression of YER044c

Experiment	Level	log ratio	Treatment [baseline]
1419	4.2	+1.7	30ug/ml Atorvastatin in 1 DMSO - 18 hr [0.12]
1420	3.6	+1.5	20ug/ml Atorvastatin in 1 DMSO - 18 hr [0.14]
1617	3.3	+1.4	20ug/ml Fluconazole - 21 hr [0.04]
1454	3.2	+1.4	8.0ug/ml Fluvastatin - 18 hr [0.13]
1537	3.1	+1.4	20ug/ml Lovastatin in 1 Ethanol - 18 hr [0.10]
1943	3.0	+1.3	15ug/ml Simvastatin in 1.5 Ethanol - 18 hr [0.13]
1623	3.0	+1.3	100ug/ml Fluconazole - 21 hr [0.04]
3456	3.0	+1.3	25ug/ml Lovastatin - 18 hr [0.20]
3455	3.0	+1.3	20ug/ml Lovastatin - 18 hr [0.20]
1611	2.9	+1.3	10ug/ml Fluconazole - 21 hr [0.04]
1553	2.7	+1.2	10ug/ml Simvastatin in 1 Ethanol - 18 hr [0.11]
3454	2.5	+1.1	10ug/ml Lovastatin - 18 hr [0.15]
1605	2.5	+1.1	5ug/ml Fluconazole - 21 hr [0.04]
3279	2.5	+1.1	0.15ug/ml Clotrimazole in 1 DMSO - 18 hr [0.13]
1455	2.4	+1.1	4.0ug/ml Fluvastatin - 18 hr [0.09]
1669	2.4	+1.1	100ug/ml Fluconazole - 8 hr [0.05]

Figure 11.

Blastp search of GenBank

Sequences producing significant alignments:	(bits)	Value
sp|P40030|YEN4_YEAST HYPOTHETICAL 17.1 KD PROTEIN IN SAH1-MEI4 ...	308	1e-83
gnl|PID|e1331605 (AL031854) conserved hypothetical protein [Sch...	110	5e-24
gi|3540193 (AC004122) Unknown protein [Arabidopsis thaliana]	46	1e-04
sp|P54142|SRB7_CAEEL SRB-7 PROTEIN >gi|1584522|prf||2123261V ch...	31	3.4

tblastn search of dbest

Sequences producing significant alignments:	(bits)	Value
gb|AA271118|AA271118 va86e12.r1 Soares mouse NML Mus musculus c...	81	9e-15
gb|AA048103|AA048103 mj23f09.r1 Soares mouse embryo NbME13.5 14...	81	9e-15
gb|AI172515|AI172515 UI-R-C2p-nu-d-02-0-UI.s1 UI-R-C2p Rattus n...	81	9e-15
gb|AA711847|AA711847 vu59b09.r1 Soares mouse mammary gland NbMM...	81	9e-15
gb|AA153659|AA153659 mq60h05.r1 Soares 2NbMT Mus musculus cDNA ...	80	2e-14
gb|W44146|W44146 mc74h02.r1 Soares mouse embryo NbME13.5 14.5 M...	80	2e-14
gb|AA269958|AA269958 va55c03.r1 Soares mouse 3NME12 5 Mus muscu...	80	2e-14
gb|W08023|W08023 mb37b04.r1 Soares mouse p3NMF19.5 Mus musculus...	78	6e-14
gb|AA014348|AA014348 mi67g10.r1 Soares mouse embryo NbME13.5 14...	73	1e-12
gb|AA272544|AA272544 va75e02.r1 Soares mouse NML Mus musculus c...	73	1e-12
gb|W13627|W13627 ma93h01.r1 Soares mouse p3NMF19.5 Mus musculus...	70	1e-11
gb|W28235|W28235 43h8 Human retina cDNA randomly primed sublibr...	70	2e-11
gb|W27040|W27040 19e6 Human retina cDNA randomly primed sublibr...	68	8e-11

Figure 12.

Mouse EST with similarity to YER044c

```
gb|AI386195|AI386195 mq60h05.y1 Soares 2NbMT Mus musculus cDNA clone
           IMAGE:583161 5' similar to SW:YEN4_YEAST P40030 HYPOTHETICAL
           17.1 KD PROTEIN YER044c. ;, mRNA sequence
           [Mus musculus]
           Length = 455

 Score = 81.5 bits (198), Expect = 6e-15
 Identities = 40/114 (35%), Positives = 68/114 (59%)
 Frame = +3

Query: 23  LPKWLLFISIVSVFNSIQTYVSGLELTRKVYERKPTETTHLSARTFGTWTFISCVIRFYG 82
           L  WL+ +SI+++ N++Q++     L  K+Y  KP     L ARTFG WT +S VIR
Sbjct: 93  LRSWLVMVSIIAMGNTLQSFRDHTFLYEKLYTGKPNLVNGLQARTFGIWTLLSSVIRCLC 272

Query: 83  AMYLNEPHIFELVFMSYMVALFHFGSELLIFRTCKLGKGFMGPLVVSTTSLVWM 136
           A+ ++   ++ +   ++++AL HF SEL +F T     G + PL+V++ S++ M
Sbjct: 273 AIDIHNKTLYHITLWTFLLALXHFLSELFVFGTAAPTVGVLAPLMVASFSILGM 434
```

Human EST with similarity to YER044c

```
gb|W28235|W28235 43h8 Human retina cDNA randomly primed sublibrary
           Homo sapiens cDNA.
           Length = 839

 Score = 69.9 bits (168), Expect = 2e-11
 Identities = 33/94 (35%), Positives = 55/94 (58%)
 Frame = +1

Query: 23  LPKWLLFISIVSVFNSIQTYVSGLELTRKVYERKPTETTHLSARTFGTWTFISCVIRFYG 82
           L  WL+ +SI+++ N++Q++     L  K+Y  KP     L ARTFG WT +S VIR
Sbjct: 112 LRSWLVMVSIIAMGNTLQSFRDHTFLYEKLYTGKPNLVNGLQARTFGIWTLLSSVIRCLC 291

Query: 83  AMYLNEPHIFELVFMSYMVALFHFGSELLIFRTC 116
           A+ ++   ++ +   ++++AL HF SEL +   C
Sbjct: 292 AIDIHNKTLYHITLWTFLLALGHFLSELFVLWNC 393
```

Figure 13.

Rat EST with similarity to YER044c

```
gb|AI172515|AI172515 UI-R-C2p-nu-d-02-0-UI.s1 UI-R-C2p Rattus
           norvegicus cDNA clone UI-R-C2p-nu-d-02-0-UI 3', mRNA
           sequence [Rattus norvegicus]
           Length = 475

 Score = 80.8 bits (196), Expect = 1e-14
 Identities = 40/114 (35%), Positives = 68/114 (59%)
 Frame = -3

Query: 23  LPKWLLFISIVSVFNSIQTYVSGLELTRKVYERKPTETTHLSARTFGTWTFISCVIRFYG 82
           L  WL+ +SI+++ N++Q++     L  K+Y  KP     L ARTFG WT +S VIR
Sbjct: 404 LRSWLVMVSIIAMGNTLQSFRDHTFLYEKLYTGKPNLVNGLQARTFGIWTLLSSVIRCLC 225

Query: 83  AMYLNEPHIFELVFMSYMVALFHFGSELLIFRTCKLGKGFMGPLVVSTTSLVWM 136
           A+ ++   ++ +   ++++AL HF SEL +F T     G + PL+V++ S++ M
Sbjct: 224 AIDIHNKTLYHITLWTFLLALGHFLSELFVFGTAAPTVGVLAPLMVASFSILGM 63
```

Figure 14.

YLR100w

GenBank No.	1360483
Chromosome	XII
Protein	347 amino acids
	39,725 Daltons
Comments:	unknown function; see S. Huang et al., Biochemistry, 26, pp. 8242-46 (1987)

Figure 15.

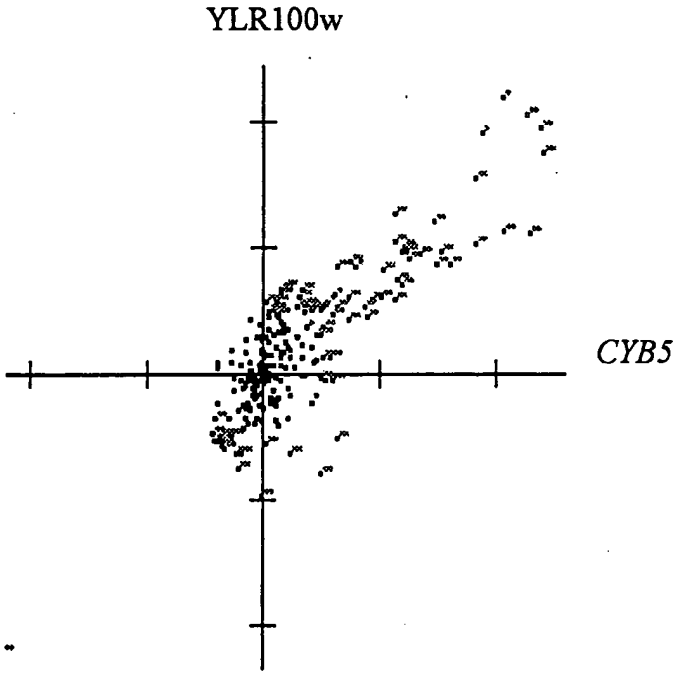


Figure 16.

Treatments Causing Highest Expression of YLR100w

Experiment	Level	Treatment [baseline]
6092	8.3	20ug/ml Lovastatin in 1 Ethanol [ABY12.1] - 24 hr [0.15]
8717	6.7	10ug/ml Simvastatin in 1 DMSO [ABY12.1] - 24 hr [0.14]
6093	6.3	10ug/ml Lovastatin in 1 Ethanol [ABY12.1] - 24 hr [0.16]
8716	6.1	7.5ug/ml Simvastatin in 1 DMSO [ABY12.1] - 24 hr [0.13]
8715	4.9	5ug/ml Simvastatin in 1 DMSO [ABY12.1] - 24 hr [0.12]
6094	4.4	5ug/ml Lovastatin in 1 Ethanol [ABY12.1] - 24 hr [0.13]
8705	2.7	[erg11 - ABY210 regulated (100)] - 24 hr [0.17]
6088	2.6	0.1ug/ml Sulconazole in 1 DMSO [ABY12.1] - 24 hr [0.12]
8341	2.5	0.025ug/ml Miconazole in 1 DMSO [ABY12.1] - 24 hr [0.15]
8460	2.4	0.1ug/ml Clotrimazole in 1 DMSO [ABY12.1] - 24 hr [0.12]
8462	2.3	0.135ug/ml Clotrimazole in 1 DMSO [ABY12.1] - 24 hr [0.17]
8461	2.3	0.12ug/ml Clotrimazole in 1 DMSO [ABY12.1] - 24 hr [0.14]
8342	2.3	0.03ug/ml Miconazole in 1 DMSO [ABY12.1] - 24 hr [0.19]
8703	2.1	[erg11 - ABY210 regulated (80)] - 24 hr [0.14]
8340	2.0	0.02ug/ml Miconazole in 1 DMSO [ABY12.1] - 24 hr [0.12]
8463	2.0	0.15ug/ml Clotrimazole in 1 DMSO [ABY12.1] - 24 hr [0.25]
8701	1.9	[erg11 - ABY210 regulated (60)] - 24 hr [0.14]

Figure 17.

Blastp search of GenBank

Sequences producing significant alignments:	Score (bits)	E Value
pir||S64936 probable membrane protein YLR100w - yeast (Saccharo...	668	0.0
emb|CAA21246| (AL031852) short-chain dehydrogenase [Schizosacch...	183	1e-45
dbj|BAA13878| (D89217) similar to Saccharomyces cerevisiae L800...	182	3e-45
emb|CAA75742| (Y15733) 17-beta-hydroxysteroid dehydrogenase typ...	85	9e-16
gi|1397235 (U44803) ovarian-specific protein [Rattus norvegicus]	84	1e-15
emb|CAB07971| (Z93941) YuxA [Bacillus subtilis] >gi|2635794|emb...	46	5e-04
emb|CAA19277| (AL023705) hypothetical protein [Schizosaccharomy...	43	0.004
dbj|BAA19567| (AB002410) 17-beta-hydroxysteroid dehydrogenase [...	39	0.046
gi|1086892 (U41277) similar to the insect-type alcohol dehydrog...	38	0.079
pir||S56475 hypothetical protein f261a - Escherichia coli >gi|5...	38	0.10
emb|CAA63039| (X91985) glycoprotein 100 [gallid herpesvirus 1]	38	0.10
gb|AAD20218| (AF100931) carbonyl reductase/20beta-hydroxysteroi...	38	0.10

tblastn search of dbest

Sequences producing significant alignments:	Score (bits)	E Value
gb|AI226514|AI226514 uj07d08.y1 Sugano mouse liver mlia Mus mus...	63	5e-09
gb|AI528381|AI528381 ui96g06.y1 Sugano mouse liver mlia Mus mus...	52	1e-05
gb|R92053|R92053 yp96c01.r1 Homo sapiens cDNA clone 195264 5'.	44	0.003
gb|AI472243|AI472243 tj86g08.x1 Soares_NSF_F8_9W_OT_PA_P_S1 Hom...	37	0.36
gb|AI321571|AI321571 d9f02nm.f1 Neurospora crassa morning cDNA ...	34	3.1
gb|AI211149|AI211149 o0a06a1.r1 Aspergillus nidulans 24hr asexu...	32	9.1
gb|AA219246|AA219246 zq16h06.r1 Stratagene fetal retina 937202 ...	32	9.1

Figure 18.

Alignment of YLR100w to Mammalian ESTs

```
gb|AI226514|AI226514 uj07d08.y1 Sugano mouse liver mlia Mus musculus cDNA
clone
           IMAGE:1891215 5' similar to TR:Q62904 Q62904
           OVARIAN-SPECIFIC PROTEIN. ;, mRNA sequence [Mus
           musculus]           Length = 1039

 Score = 63.2 bits (151), Expect = 5e-09
 Identities = 53/223 (23%), Positives = 108/223 (47%), Gaps = 11/223 (4%)

Query: 3    RKVAIVTGTNSNLGLNIVFRLIETEDTNVRLTIVVTSRTLPRVQEVINQIKDFYNKSGRV 62
             RKV ++TG +S +GL +  RL+  +D    L + +  R L + + V + +   +  +
Sbjct: 52   RKVVLITGASSGIGLALCGRLLAEDDD---LHLCLACRNLSKARAVRDTLLASHPSA--- 213

Query: 63   EDLEIDFDYLLVDFTNMVSVLNAYYDINKKYRAINYLFVNAA---------QGIFDGIDW 113
                  +   + +D +++ SV+    ++ +K++ ++YL++NA          +  F GI +
Sbjct: 214  -----EVSIVQMDVSSLQSVVRGAEEVKQKFQRLDYLYLNAGILPNPQFNLKAFFCGI-F 375

Query: 114  IGAVKEVFTNPLEAVTNPTYKIQLVGVKSKDDMGLIFQANVFGPYYFISKILPQLTRGK- 172
                V  +FT   E +     +   G++       +F+ N+FG +  I  ++ P L
Sbjct: 376  SRNVIHMFTTA-EGILTQNDSVTADGLQE------VFETNLFGHFILIRELEPLLCHADN 534

Query: 173  -AYIVWISSIMSDPKYLSLNDIELLKTNASYEGSKRLVDLLHLATYKDLKKLGI 225
             + ++W SS  +     SL DI+  K    Y   +   DLL++A  ++ K  G+
Sbjct: 535  PSQLIWTSSRNAKKANFSLEDIQHFKGPEPYSSFQYATDLLNVAXNREFKPEGL 696


 gb|AI528381|AI528381 ui96g06.y1 Sugano mouse liver mlia Mus musculus cDNA
clone
           IMAGE:1890298 5' similar to TR:Q62904 Q62904
           OVARIAN-SPECIFIC PROTEIN. ;, mRNA sequence [Mus
           musculus]           Length = 837

 Score = 52.3 bits (123), Expect = 1e-05
 Identities = 59/260 (22%), Positives = 119/260 (45%), Gaps = 11/260 (4%)

Query: 3    RKVAIVTGTNSNLGLNIVFRLIETEDTNVRLTIVVTSRTLPRVQEVINQIKDFYNKSGRV 62
             RKV ++TG +S +GL +  RL+  +D    L + +  R L + + V + +   +  +
Sbjct: 52   RKVVLITGASSGIGLALCGRLLAEDDD---LHLCLACRNLSKARAVRDTLLASHPSA--- 213

Query: 63   EDLEIDFDYLLVDFTNMVSVLNAYYDINKKYRAINYLFVNAA---------QGIFDGIDW 113
                  +   + +D +++ SV+    ++ +K++ ++YL++NA          +  F GI +
Sbjct: 214  -----EVSIVQMDVSSLQSVVRGAEEVKQKFQRLDYLYLNAGILPNPQFNLKAFFCGI-F 375

Query: 114  IGAVKEVFTNPLEAVTNPTYKIQLVGVKSKDDMGLIFQANVFGPYYFISKILPQLTRGK- 172
                V  +FT   E +     +   G++       +F+ N+FG +  I  ++ P L
Sbjct: 376  SRNVIHMFTTA-EGILTQNDSV------TADRLQEVFETNLSCHFILIRELEPLLLHADN 534

Query: 173  -AYIVWISSIMSDPKYLSLNDIELLKTNASYEGSKRLVDLLHLATYKDLKKLGINQYVVQ 231
             + ++W SS  +     SL D +       Y   +   DLL++A  +  + G+  +
Sbjct: 535  PSQLIWTSSRNAXKANFSLEDXQHSIGPGPYSSFQYATDLLNVALNXNXNQKGLYSSRMC 714

Query: 232  PGIFTSHSFSEYLNFFTYFGMLCLFYLARLL 262
             PG+  ++          TY G+L  FYL  LL
Sbjct: 715  PGVVMTN--------MTY-GILPPFYLDVLL 780
```

Figure 19.

```
gb|R92053|R92053 yp96c01.r1 Homo sapiens cDNA clone 195264 5'.Length = 454

Score = 44.1 bits (102), Expect = 0.003
Identities = 26/84 (30%), Positives = 40/84 (46%), Gaps = 2/84 (2%)
Frame = +1

Query: 150 FQANVFGPYYFISKILPQLTRGK--AYIVWISSIMSDPKYLSLNDIELLKTNASYEGSKR 207
           F+ NVFG +  I ++ P L      + ++W SS  +     SL D +  K    Y  SK
Sbjct: 1   FETNVFGHFILIRELEPLLCHSDNPSQLIWTSSRSARKSNFSLEDFQHSKGKEPYSSSKY 180

Query: 208 LVDLLHLATYKDLKKLGINQYVVQPG 233
             DLL +A  ++  + G+   V  PG
Sbjct: 181 ATDLLSVALNRNFNQQGLYSNVACPG 258
```

Figure 19 (cont).

YER034w

GenBank No.	603267
Chromosome	V
Protein	185 amino acids
	21,186 Daltons
Comments:	unknown function; see S. Huang et al., Biochemistry, 26, pp. 8242-46 (1987)

Figure 20

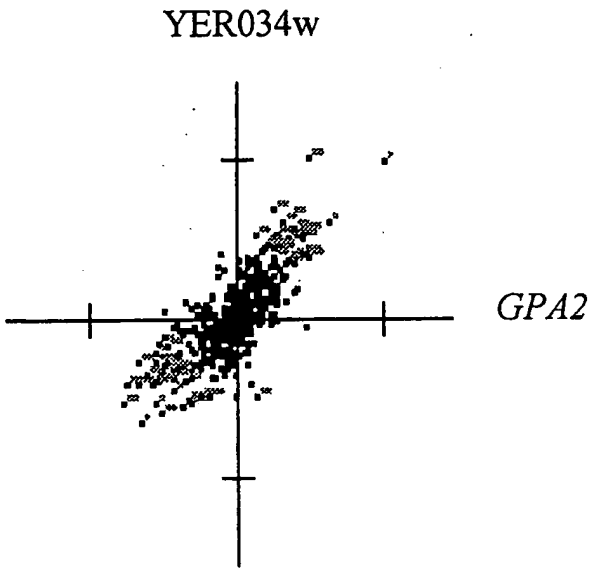


Figure 21.

Mutation of the *YER034w* Gene Leads to Increased Pseudohyphal Growth

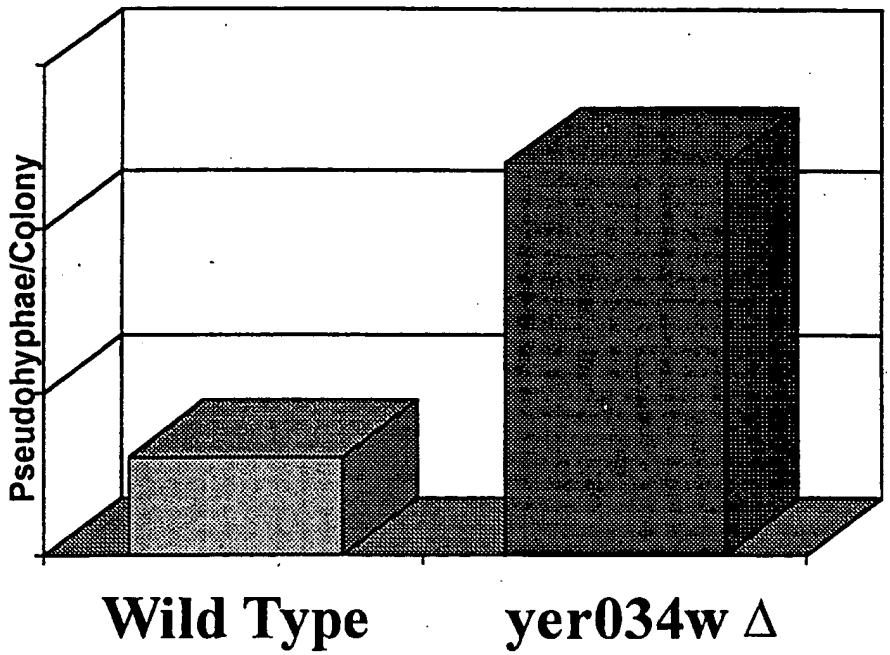


Figure 22.

YKL077w

GenBank No.	486110
Chromosome	XI
Protein	392 amino acids
	46,042 Daltons
Comments:	unknown function; potential transmembrane domain

Figure 23

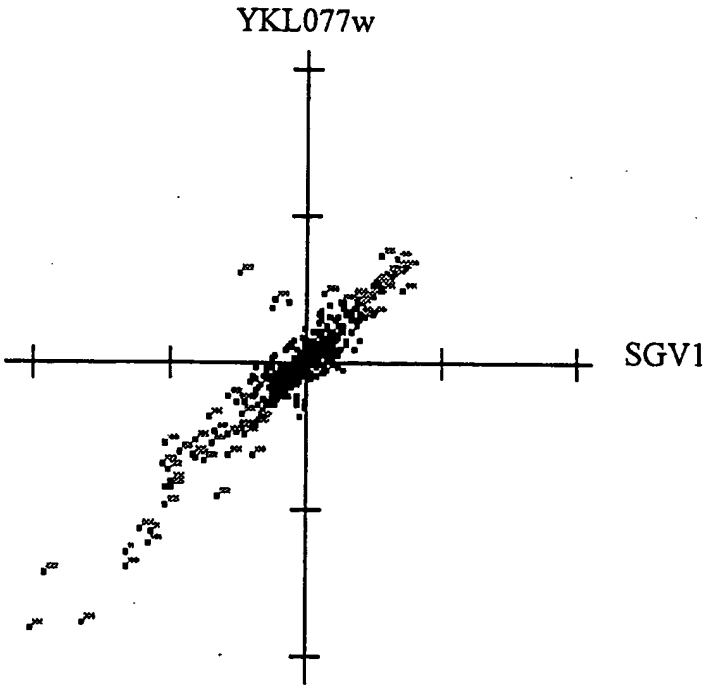


Figure 24.

Expression Correlation of YKL077w

Rank	Gene	Correlation	Exp	Function
1	YKL077w	+1.00	0.5 - 9.1	
2	SGV1	+0.92	0.7 - 14.4	CDC28/cdc2 related protein kinase
3	RHO1	+0.88	1.3 - 20.9	GTP-binding protein
4	YKL075c	+0.86	0.2 - 2.5	
5	SRA3	+0.84	0.3 - 4.6	catalytic subunit of PKA
6	RPB4	+0.84	0.3 - 7.8	subunit of RNA polymerase II
7	PKC1	+0.84	0.6 - 11.7	putative protein kinase

Figure 25.

Blastp search of GenBank

Sequences producing significant alignments:	Score (bits)	E Value
sp|P36081|YKH7_YEAST HYPOTHETICAL 46.0 KD PROTEIN IN SMY1-MUD2 ...	785	0.0
gi|1172087 (U19568) squamous cell carcinoma antigen [Homo sapie...	35	0.75
sp|P54634|POLN_LORDV NON-STRUCTURAL POLYPROTEIN [CONTAINS: RNA-...	35	0.75
sp|P29508|SCC1_HUMAN SQUAMOUS CELL CARCINOMA ANTIGEN 1 (SCCA-1)...	35	0.75
pir||I38201 squamous cell carcinoma antigen 1 - human	35	0.75
gi|3063469 (AC003981) F22O13.31 [Arabidopsis thaliana]	35	0.98
pir||S23760 polyphenolic adhesive protein - blue mussel (fragme...	34	2.2
sp|P37222|MAOC_LYCES MALATE OXIDOREDUCTASE, CHLOROPLAST (MALIC ...	32	5.0

tblastn search of dbest

Sequences producing significant alignments:	Score (bits)	E Value
gb|T38483|T38483 EST103979 Saccharomyces cerevisiae cDNA 3' end.	99	6e-20
gb|T36426|T36426 EST101359 Saccharomyces cerevisiae cDNA 3' end.	69	1e-10
gb|AA724136|AA724136 ai07b06.s1 Soares parathyroid tumor NbHPA ...	32	9.2

Figure 26.

YGR046w

GenBank No.	1323049
Chromosome	VII
Protein	385 amino acids
	44,219 Daltons
Comments:	essential gene in yeast

Figure 27

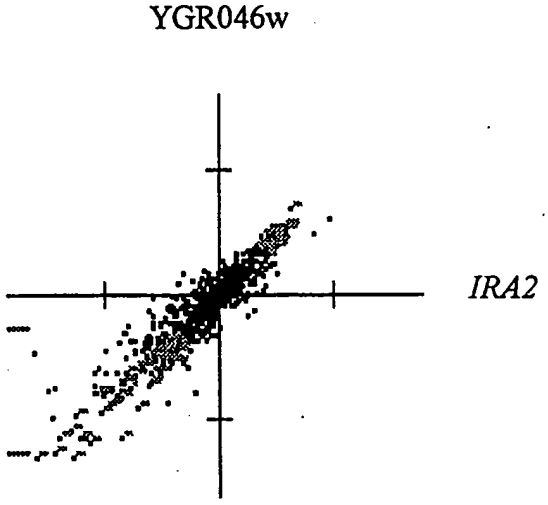


Figure 28.

Expression Correlation to YGR046w

Gene	Correlation	Levels	Function
YGR046w	+1.00	0.9 - 10.1	similar to phage 1C ANTP-139 protein PIR:S46430
IRA2	+0.90	0.3 - 5.4	GTPase activating protein, neurofibromin homolog
RLR1	+0.89	0.8 - 6.1	Regulatory protein, post-transcription initiation
NUT1	+0.85	0.4 - 3.2	Negative regulator of HO endonuclease promoter
SRO7	+0.84	0.3 - 4.9	Drosphila tumor suppressor homolog, rho3 suppressor
DST1	+0.84	0.5 - 4.5	RNA polymerase II elongation factor
MTR3	+0.84	1.4 - 11.8	mRNA transport
TPD3	+0.82	2.6 - 22.0	protein phosphatase (PP2A regulatory subunit)
SYF3	+0.80	0.1 - 2.0	similar to Drosophila probable cell cycle control
MEX67	+0.78	1.2 - 14.1	INvolved in nuclear mRNA export, binds both poly(A)
YNK1	+0.78	1.1 - 24.2	Nucleoside diphosphate kinase
MPD2	+0.78	0.3 - 6.0	protein disulfide isomerase related protein
BEM2	+0.77	0.7 - 10.7	Rho-type GTPase activating protein (GAP

Figure 29.

Treatments Causing the Most Significant Changes in Expression of YGR046w

Experiment	Levels	Log ratio	Treatment [baselines]
11757	10.1/5.0	0.9	20ug/ml 2,4-Dinitrophenol in 1% DMSO [ABY12 6144C yx-101] - 24 hr [0.19/0.05]
7571	7.3/3.9	0.8	2000ug/ml p-Aminosalicylic Acid in 2% DMSO [ABY12.1] - 24 hr [0.21/0.08]
10815	7.7/4.3	0.8	1500ug/ml Acetylsalicylic Acid in 1% DMSO [ABY12] - 24 hr [0.20/0.05]
10482	8.2/4.6	0.7	600ug/ml Sodium Nitrite in 1% DMSO (ABY12.1] - 24 hr [0.23/0.07]
10819	7.2/4.2	0.7	900ug/ml Acetylsalicylic Acid 10 ug/ml Methotrexate in 1% DMSO [ABY12] - 24 hr [0.14/0.05]
7877	8.2/4.8	0.7	1200ug/ml p-Aminosalicylic Acid in 1% DMSO [ABY12.1] - 24 hr [0.22/0.09]
10814	7.3/4.3	0.7	1200ug/ml Acetylsalicylic Acid in 1% DMSO [ABY12] - 24 hr [0.15/0.05]
10822	7.0/4.2	0.7	300ug/ml Acetylsalicylic Acid 20 ug/ml Methotrexate in 1% DMSO [ABY12] - 24 hr [0.09/0.05]
9107	6.3/3.8	0.7	550ug/ml Thiourea in 1% DMSO [ABY12.1] - 24 hr [0.12/0.05]
7573	6 5/3.9	0.7	2130ug/ml p-Aminosalicylic Acid in 2% DMSO [ABY12.1] - 24 hr [0.21/0.08]
10481	7.6/4.6	0.7	500ug/ml Sodium Nitrite in 1% DMSO [ABY12.1) - 24 hr [0.18/0.07]
8362	5.6/3.4	0.7	6ug/ml 8-Hydroxyquinoline in 1% DMSO [ABY12.1] - 24 hr [0.07/0.06]
9613	6.2/3.8	0.7	0.1ug/ml Azoxystrobin in 1% DMSO [ABY12.1] - 24 hr [0.15/0.06]

Figure 30.

Experiment	Levels	Log ratio	Treatment [baselines]
10983	2.2/5.2	-0.7	50ug/ml Maleimide in 1% DMSO [ABY12] - 24 hr [0.14/0.07]
8737	2 0/5.0	-0.7	2ug/ml 5-Fluorocytosine in 1% DMSO [ABY12.1] - 24 hr (0.12/0.06)
8263	1.8/4.4	-0.7	4.5ug/ml Dimethyl Sulfoxide in 1% DMSO [ABY12.1] - 24 hr [0.18/0.6]
11679	1.6/4.0	-0.7	600ug/ml Tricyclazole in 2% DMSO [ABY 12 6144C yx-101] - 24 hr (0.33/0.05]
8435	1.8/4.6	-0.8	20ug/ml Benomyl in 1% DMSO [ABY12.1] - 24 hr [027/0.06]
10802	1.6/4 2	-0.8	20ug/ml Cumene Hydroperoxide in 1% DMSO [ABY12] - 24 hr [0.11/0.06]
9633	1.4/3.7	-0.8	80ug/ml Pyrimethanil in 1% DMSO [ABY12.1] - 24 hr [0.27/0.06]
9340	1.3/4.0	-0.9	0.03ug/ml Cycloheximide in 1% DMSO [ABY12.1] - 24 hr [0.25/0.06]
8354	-1.3/4.1	-1	100ug/ml Quinacrine in 1% DMSO (ABY12.1] - 24 hr [0.10/0.06]
11774	1.5/4.7	-1	5ug/ml Sodium azide in 1% DMSO [ABY 12 6144C yx-101] - 24 hr [0.13/0.06]
9588	1.1/3.5	-1	600ug/ml Hydrogen Peroxide in 1% DMSO [ABY12.1] - 24 hr [0.14/0.08]
10574	1.7/5.3	-1	300ug/ml Pyroquilon in 1% DMSO [ABY12] - 24 hr [0.34/0.10]
12366	1.4/4.7	-1	0.2ug/ml Thimerosal in 1% DMSO [ABY 12 6144C yx-101] - 24 hr [0.10/0.07]
8429	1.1/3.8	-1.1	250ug/ml Benfluorex Hydrochloride in 1% DMSO [ABY12.1] - 24 hr [0.15/0.06]
11775	1.3/4.7	-1.1	6ug/ml Sodium azide in 1% DMSO [ABY 12 6144C yx-101] - 24 hr [0.21/0.06]
12327	1.4/5.1	-1.1	1000ug/ml Benzimidazole in 1% DMSO [ABY12 6144C yx-101] - 24 hr [0.16/0.07]
11595	0.9/4.7	-1.5	10ug/ml Sodium azide in 1% DMSO [ABY 12 6144C yx-101] - 24 hr [0.27/0.05]

Figure 30 (cont).

YJR041c

GenBank No.	1015693
Chromosome	X
Protein	1173 amino acids 135,096 Daltons
Comments:	essential gene in yeast; contains a leucine zipper; potential transmembrane domain

Figure 31

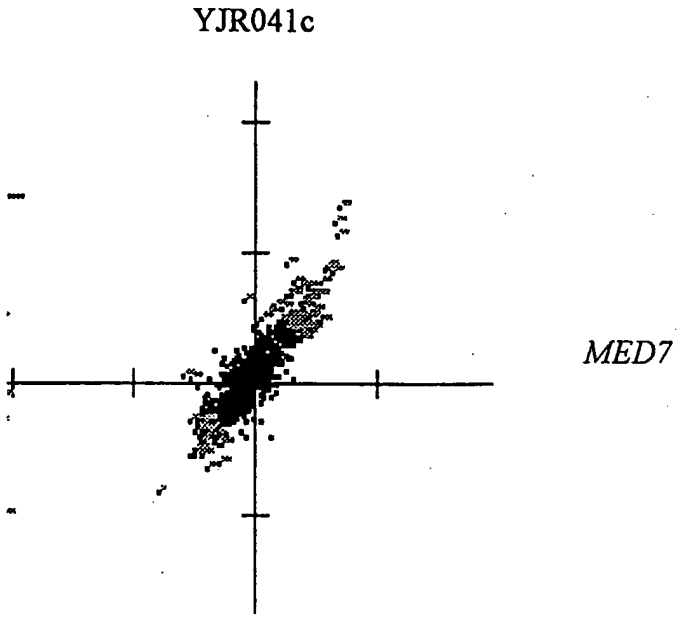


Figure 32.

Correlation to YJR041c

Gene	Correlation	Exp	Function
YJR041c	+1.00	0.2 - 1.7	similar to Podospora anserina NADH dehydrogenase
MED7	+0.83	0.3 - 0.9	Stoichiometric member of mediator complex
SNP2	+0.82	0.5 - 2.0	snRNP G protein (the homologue of the human Sm-G)
RPA43	+0.82	0.5 - 2.4	DNA-dependent RNA polymerase I subunit A43
SLS1	+0.81	0.3 - 1.5	73 kDa mitochondrial integral membrane protein
SEC53	+0.80	0.7 - 4.1	phosphomannomutase
EMP47	+0.76	0.3 - 1.5	47 kDa type I transmembrane protein
POP4	+0.76	0.2 - 1.0	RNase P and RNase MRP subunit
RPL1A	+0.75	1.4 - 5.8	homolog of bacterial ribosomal proteins of L1 family
RPC53	+0.74	0.2 - 0.9	RNA polymerase III (C) subunit
SKI6	+0.72	0.5 - 2.4	ExtraCellular Mutant; ribosomal RNA processing
UPF3	+0.72	0.1 - 0.4	Stimulates decay of mRNAs with premature stop codons
TAF19	+0.71	0.1 - 0.4	TFIID subunit
RPL37B	+0.71	1.5 - 7.8	60S ribosomal protein YL35
GCD10	+0.71	0.5 - 2.3	RNA-binding subunit, translation initiation factor 3

Figure 33.

Blastp search of GenBank

Sequences producing significant alignments:	Score (bits)	E Value
sp|P47108|YJ11_YEAST HYPOTHETICAL 135.1 KD PROTEIN IN GEF1-NUP8...	2238	0.0
emb|CAA89570| (Z49542) ORF YJR041c [Saccharomyces cerevisiae]	2127	0.0
sp|Q09804|YAB2_SCHPO HYPOTHETICAL 150.5 KD PROTEIN C2G11.02 IN ...	53	8e-06
emb|CAA91167| (Z54354) hypothetical protein [Schizosaccharomyce...	53	8e-06
gi|3929312 (AF100426) fimbriae-associated protein Fap1 [Strepto...	43	0.012
gi|2688777 (AE001181) exonuclease SbcC (sbcC) [Borrelia burgdor...	38	0.51
gi|2462828 (AF000657) hypothetical protein [Arabidopsis thaliana]	35	2.6
pir||S43557 coiled coil protein B0284.1 - Caenorhabditis elegan...	35	2.6
gi|2315501 (AF016451) No definition line found [Caenorhabditis ...	35	2.6
sp|P13496|DYNA_DROME 150 KD DYNEIN-ASSOCIATED POLYPEPTIDE (DP-1...	34	5.8
sp|Q58042|Y625_METJA HYPOTHETICAL ATP-BINDING PROTEIN MJ0625 >g...	34	5.8
gb|AAD18581| (AE001626) ClpC Protease [Chlamydia pneumoniae]	34	5.8
gi|3098583 (AF049495) gag polyprotein [Human immunodeficiency v...	34	7.5
sp|P44581|NHAA_HAEIN NA(+)/H(+) ANTIPORTER 1 >gi|1075053|pir||C...	33	9.9
gi|2062752 (U92845) kinesin motor protein [Ustilago maydis]	33	9.9

tblastn search of dbest

Sequences producing significant alignments:	Score (bits)	E Value
gb|AI201151|AI201151 qf64h07.x1 Soares_testis_NHT Homo sapiens ...	36	3.7
gb|AA747649|AA747649 nx77g11.s1 NCI_CGAP_Ew1 Homo sapiens cDNA ...	34	8.4
gb|AI248270|AI248270 qh75g09.x1 Soares_fetal_liver_spleen_1NFLS...	34	8.4

Figure 34.

HES1

GenBank No.	1420543
Chromosome	XV
Protein	433 amino acids
	49,502 Daltons
Comments:	implicated in ergosterol pathways; related to human oxysterol binding protein

Figure 35

Expression Correlation to HES1

Gene	Correlation	Exp	Function
HES1	+1.00	0.1 - 7.2	homology to human oxysterol binding protein
ERG2	+0.90	0.1 - 5.3	C-8 sterol isomerase
PAU5	+0.89	0.1 - 4.7	member of seripauperin protein/gene family
ERG7	+0.83	0.2 - 3.0	lanosterol synthase
CYB5	+0.83	0.4 - 17.8	cytochrome b5
YJL105w	+0.81	0.1 - 4.7	similar to Ykr029p
YER044c	+0.79	0.3 - 3.7	
ERG11	+0.79	0.3 - 13.0	cytochrome P450 lanosterol 14a-demethylase
HEM14	+0.76	0.1 - 1.3	protoporphyrinogen oxidase
ERG9	+0.76	0.8 - 8.8	squalene synthetase
TIR1	+0.74	0.2 - 6.8	cold-shock induced - serine-alanine-rich
ERG8	+0.70	0.3 - 6.0	phosphomevalonate kinase
ERG6	+0.69	0.5 - 9.6	SAM: delta 24-methyltransferase

Figure 36.

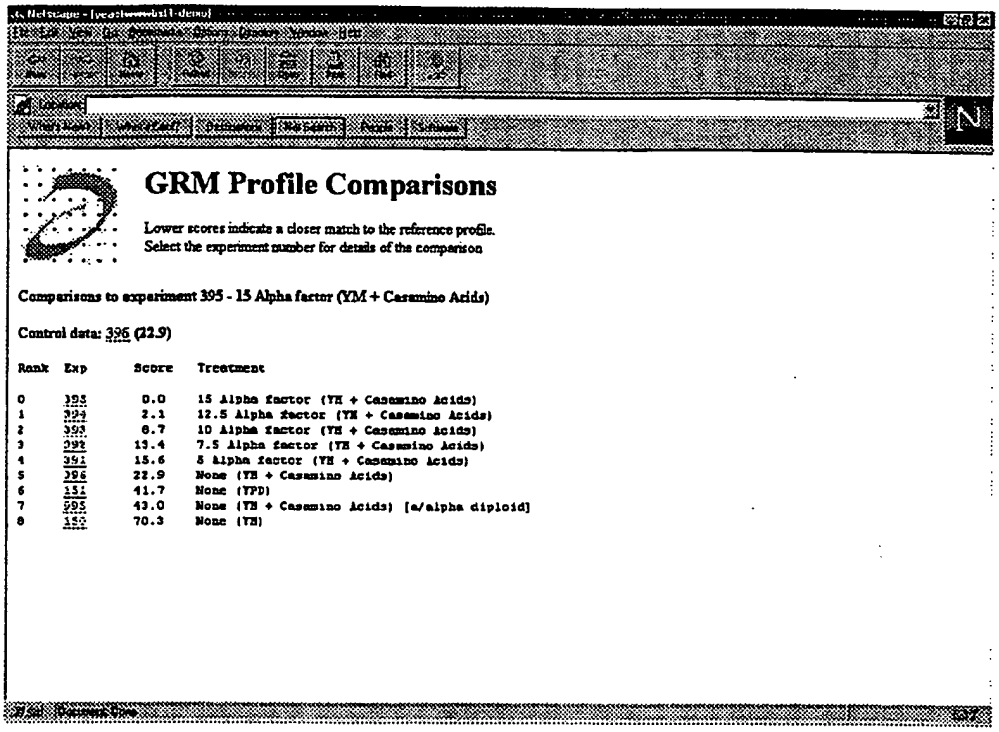
Treatments that Induce the *HES1* Reporter

Experiment	Levels	Log ratio	Treatment [baselines]
9923	7.2/0.1	4.1	20ug/ml Simvastatin in 1% DMSO [ABY12.1] - 24 hr [0.19/0.07]
9930	4.6/0.1	3.6	40ug/ml Lovastatin in 1% DMSO [ABY12.1] - 24 hr [0.18/0.07]
9708	4.5/0.1	3.6	15ug/ml Atorvastatin in 1% DMSO [ABY12.1] - 24 hr [0.17/0.05]
9709	4.4/0.1	3.6	20ug/ml Atorvastatin in 1% DMSO [ABY12.1] - 24 hr [0.16/0.05]
9924	4.2/0.1	3.6	40ug/ml Simvastatin in 1% DMSO [ABY12.1] - 24 hr [0.27/0.07]
6092	4.4/0.1	3.5	20ug/ml Lovastatin in 1% Ethanol [ABY12.1] - 24 hr [0.16/0.08]
9909	3.8/0.1	3.5	10ug/ml Fluvastatin in 1% DMSO [ABY12.1] - 24 hr [0.18/0.07]
9707	3.7/0.1	3.5	10ug/ml Atorvastatin in 1% DMSO [ABY12.1] - 24 hr [0.15/0.05]
8465	3.0/0.1	3.2	0.03ug/ml Econazole in 1% DMSO [ABY12.1] - 24 hr [0.26/0.06]
9922	2.8/0.1	3.2	10ug/ml Simvastatin in 1% DMSO [ABY12.1] - 24 hr [0.14/0.07]
8463	2.7/0.1	3.1	0.15ug/ml Clotrimazole in 1% DMSO [ABY12.1] - 24 hr [0.25/0.06]
9797	2.6/0.1	3.1	6ug/ml Fluvastatin in 1% DMSO [ABY12.1] - 24 hr [0.12/0.05]
6093	2.7/0.1	3	10ug/ml Lovastatin in 1% Ethanol [ABY12.1] - 24 hr [0.18/0.08]
8717	2.4/0.1	3	10ug/ml Simvastatin in 1% DMSO [ABY12.1] - 24 hr [0.14/0.06]

Figure 37.

Experiment	Levels	Log ratio	Treatment [baselines]
9706	2.2/0.1	2.9	5ug/ml Atorvastatin in 1% DMSO [ABY12.1] - 24 hr [0.11/0.05]
9908	2.1/0.1	2.9	6ug/ml Fluvastatin in 1% DMSO [ABY12.1] - 24 hr [0.14/0.07]
9929	2.1/0.1	2.8	20ug/ml Lovastatin in 1% DMSO [ABY12.1] - 24 hr [0.16/0.07]
8716	2.1/0.1	2.8	7.5ug/ml Simvastatin in 1% DMSO [ABY12.1] - 24 hr [0.13/0.06]
8461	1.8/0.1	2.7	0.12ug/ml Clotrimazole in 1% DMSO [ABY12.1] - 24 hr [0.14/0.06]
8342	1.8/0.1	2.7	0.03ug/ml Miconazole in 1% DMSO [ABY12.1] - 24 hr [0.19/0.06]
9796	1.7/0.1	2.7	4ug/ml Fluvastatin in 1% DMSO [ABY12.1] - 24 hr [0.10/0.05]
8462	1.7/0.1	2.7	0.135ug/ml Clotrimazole in 1% DMSO [ABY12.1] - 24 hr [0.17/0.06]
6088	1.4/0.1	2.6	0.1 ug/ml Sulconazole in 1% DMSO [ABY12.1] - 24 hr [0.12/0.07]
8341	1.5/0.1	2.5	0.025ug/ml Miconazole in 1% DMSO [ABY12.1] 24 hr [0.15/0.06]
8460	1.3/0.1	2.4	0.1ug/ml Clotrimazole in 1% DMSO [ABY12.1] - 24 hr [0.12/0.06]
8715	1.3/0.1	2.3	5ug/ml Simvastatin in 1% DMSO [ABY12.1] - 24 hr [0.12/0.06]
9921	1.1/0.1	2.3	5ug/ml Simvastatin in 1% DMSO [ABY12.1] - 24 hr [0.18/0.07]

Figure 37 (cont).



GRM Profile Comparisons

Lower scores indicate a closer match to the reference profile.
Select the experiment number for details of the comparison

Comparisons to experiment 395 - 15 Alpha factor (YM + Casamino Acids)

Control data: 396 (22.9)

Rank	Exp	Score	Treatment
0	395	0.0	15 Alpha factor (YE + Casamino Acids)
1	394	2.1	12.5 Alpha factor (YE + Casamino Acids)
2	393	6.7	10 Alpha factor (YE + Casamino Acids)
3	392	13.4	7.5 Alpha factor (YE + Casamino Acids)
4	391	15.6	5 Alpha factor (YE + Casamino Acids)
5	396	22.9	None (YE + Casamino Acids)
6	151	41.7	None (YPD)
7	995	43.0	None (YE + Casamino Acids) [a/alpha diploid]
8	150	70.3	None (YE)

Figure 38

FIGURE 39. *YJL105w* DNA Sequence

Sequence contains 1200bp of 5' promoter sequence.
Symbols: 1 to: 2883 from: chr10.gcg ck: 4711,
223552 to: 226434
Chromosome X Sequence
EMBO J. 15:2031-2049 [8641269] (1996) Complete nucleotide sequence of Saccharomyces cerevisiae chromosome X.
Galibert, F., Alexandraki, D., Baur, A., Boles, E., Chalwatzis, N., Chuat, J. C., Coster, F., Cziepluch, C., De Haan, M., Domdey, H., . . .

gcgseq.tmp.4454 Length: 2883 March 26, 1999 16:51 Type: N Check: 6274 ..

```
   1  TGGAAAAGCT CACTGTGAGG TTCCTTGGAG CCAATAGTAA TACAGCACAA
  51  TCCAAGGAAA AATCTGGCCT ATATGCAAGG AAGGAGAGAT AGTCAAAAGC
 101  ATTCTTTCCC CTAGAAGTTG GTGCATATAT GGCATCGTTA AAACATATTA
 151  CCCCCAAAAT TTCTTCTCTA AACGATGTGC TTGGCCTTTG TTTTGGTTTT
 201  TGATGTCGGT CGTTTGAGGC CCCTTGCGGA AAATCGAGAT CGCCGAATGG
 251  CACGCGAGGG AAGGGAAATA AGGTTTAAAG GCACTGAAAC AATAGGCAAG
 301  AAGTAGGCGA GAGCCGACAT ACGAGACTAA TGTGTCCGCG TTTCTAAGGC
 351  CACTTTTCAA TGAAACGGAT ATTGATATGC TAGTAAAAGG ACGAGCTCAA
 401  GAGCGAAAAT ATAAGTAAAG AATTCGAGTG CACTTGTCTC CATGCAGCAA
 451  GATTTCATAT GAGTCTTTTT TATCTTTTTA CTTTTTACAT TACACGATAT
 501  GCACTTTATG AAAATTTAAC GAGGTTGGAA GCCGGATAAT CAACCAAAAT
 551  CAGGCACGAA GGCACACTCG TATATGCATG TTGTTGAAAC TCTGTTACGC
 601  TGAACTAACA ATCACACATG TAGAGGTCAC CGGGAAAAGT TGCGACCCCA
 651  TGGAAGGTCG ATCTCTTCGT TTGGCTTTGC TTGGCTGGCG GCATTGCGCT
 701  TCTTCGCTTA TACCCGTCTC TTGACGCTCG AGCTCGTTCA TTGAGATACC
 751  TTTATTCTTG CACATTTTCT GGCTTTTTTC GCTACTCGGG TACATGTAAT
 801  CATGCACACA GAAGGTGCTG TAGGGTGAAA GTTCCTTTGT GCTGTCGTTT
 851  GTTTTTAATG CCAAACTTTC TGGTGATCAA TAACCACCTC TTTTTCCTTC
 901  AGGAAACCTT ATTATTGTTC TTGGATAGTA CTAGGAAGTA TATAAGGAAC
 951  CTCGATTTTG GTATTGCACG GCTATACACA TCTAAGAAAC TTTGTATAAA
1001  AGGTGGCTAC CCTATTCATA GCTTGATATC AATAGGCCAT CTCATCACTT
1051  TTTATTGAAA AGGAAAGGAG GGAAATATAT CTGATTCAAA TTACTTGTTT
1101  GCTTCTCTTT AAGACAAAAG CATAGATAAT TTCAGCGTGG AACGCCGGAA
1151  TAAGATTGGT ACCCTCGTCA GAAAGTTACA AATACCGCTT CATCTTCAAA
1201  ATGACTTCAC CGGAATCACT ATCTTCTCGT CATATCAGGC AAGGAAGGAC
1251  ATACACAACC ACAGACAAGG TCATATCGCG GTCGTCGTCG TACTCATCTA
1301  ATAGTTCAAT GTCTAAAGAT TACGGCGATC ACACACCCTT GTCCGTCAGC
1351  AGTGCAGCTT CAGAGACATT ACCCTCACCT CAGTATATGC CGATAAGGAC
1401  ATTCAATACA ATGCCTACAG CTGGCCCAAC GCCTTTACAT TTATTTCAAA
1451  ATGACAGGGG CATTTTCAAC CATCATTCTT CATCAGGCTC ATCAAAAACG
1501  GCATCAACAA ATAAAAGAGG AATAGCAGCA GCAGTAGCAT TGGCAACTGC
1551  TGCCACCATA CCATTTCCAC TGAAAAAACA GAATCAAGAT GATAATTCCA
1601  AGGTCTCGGT AACACACAAT GAATCATCGA AAGAAAATAA AATTACACCC
1651  TCCATGAGAG CAGAAGATAA CAAACCTAAA AATGGTTGCA TCTGCGGTTC
1701  AAGTGACTCC AAGGATGAGT TGTTTATACA GTGTAACAAA TGTAAAACGT
1751  GGCAGCACAA GTTATGTTAT GCTTTCAAAA AATCAGATCC AATAAAAAGA
```

```
1801  GATTTTGTTT GCAAAAGATG TGACAGTGAT ACGAAAGTGC AGGTTAATCA
1851  AGTAAAACCA ATGATATTCC CTAGAAAAAT GGGAGATGAG CGATTATTTC
1901  AATTTTCATC CATAGTGACA ACTTCAGCAT CGAACACAAA TCAGCATCAA
1951  CAGTCTGTGA ATAACATAGA GGAACAGCCC AAGAAACGTC AACTTCATTA
2001  TACCGCCCCA ACAACTGAAA ATAGCAATAG TATACGGAAA AAATTGAGGC
2051  AAGAAAAACT GGTAGTATCA AGCCACTTTC TGAAGCCACT ACTGAATGAG
2101  GTAAGTTCTT CCAATGACAC GGAATTCAAA GCAATAACAA TATCAGAGTA
2151  TAAGGACAAA TATGTTAAGA TGTTTATTGA TAACCATTAT GATGACGATT
2201  GGGTTGTTTG TTCTAACTGG GAAAGCTCAA GGTCAGCTGA CATCGAGGTA
2251  AGAAAATCAT CAAATGAAAG AGATTTTGGA GTCTTCGCTG CAGATTCTTG
2301  TGTTAAAGGT GAGCTAATTC AAGAATATTT GGGCAAAATT GATTTTCAAA
2351  AAAATTATCA GACAGATCCA AATAATGACT ATCGTTTGAT GGGAACGACA
2401  AAACCTAAAG TACTTTTTCA TCCACATTGG CCTTTATATA TAGACTCTCG
2451  AGAAACAGGC GGATTAACAA GATACATAAG ACGGAGTTGT GAGCCCAATG
2501  TGGAACTAGT AACGGTAAGA CCGCTTGACG AAAAACCAAG AGGAGATAAT
2551  GATTGTAGAG TTAAATTTGT TTTAAGGGCT ATAAGAGATA TTCGTAAGGG
2601  AGAAGAGATA AGCGTAGAAT GGCAATGGGA TTTGAGAAAT CCTATTTGGG
2651  AGATAATAAA TGCATCTAAA GATTTGGATT CCCTACCGGA TCCCGACAAG
2701  TTCTGGTTGA TGGGGTCAAT AAAGACTATT TTAACAAATT GTGATTGTGC
2751  ATGTGGGTAC TTGGGCCATA ATTGTCCAAT AACTAAAATC AAAAACTTTT
2801  CTGAAGAATT CATGAGGAAT ACGAAGGAAT CCCTATCTAA TAAATCTTAC
2851  TTTAATACAA TAATGCACAA CTGTAAGCCA TAA
```

FIGURE 39 (cont).

FIGURE 40. *YJL105W* Protein Sequence

EMBO J. 15:2031-2049 [8641269] (1996) Complete nucleotide sequence of Saccharomyces cerevisiae chromosome X.

Galibert, F., Alexandraki, D., Baur, A., Boles, E., Chalwatzis, N., Chuat, J. C., Coster, F., Cziepluch, C., De Haan, M., Domdey, H., Durand, P., Entian, K. D., Gatius, M., Goffeau, A., Grivell, L. A., et al.

YJL105W Length: 560 March 26, 1999 16:52 Type: P Check: 103 ..

```
   1  MTSPESLSSR HIRQGRTYTT TDKVISRSSS YSSNSSMSKD YGDHTPLSVS
  51  SAASETLPSP QYMPIRTFNT MPTAGPTPLH LFQNDRGIFN HHSSSGSSKT
 101  ASTNKRGIAA AVALATAATI PFPLKKQNQD DNSKVSVTHN ESSKENKITP
 151  SMRAEDNKPK NGCICGSSDS KDELFIQCNK CKTWQHKLCY AFKKSDPIKR
 201  DFVCKRCDSD TKVQVNQVKP MIFPRKMGDE RLFQFSSIVT TSASNTNQHQ
 251  QSVNNIEEQP KKRQLHYTAP TTENSNSIRK KLRQEKLVVS SHFLKPLLNE
 301  VSSSNDTEFK AITISEYKDK YVKMFIDNHY DDDWVVCSNW ESSRSADIEV
 351  RKSSNERDFG VFAADSCVKG ELIQEYLGKI DFQKNYQTDP NNDYRLMGTT
 401  KPKVLFHPHW PLYIDSRETG GLTRYIRRSC EPNVELVTVR PLDEKPRGDN
 451  DCRVKFVLRA IRDIRKGEEI SVEWQWDLRN PIWEIINASK DLDSLPDPDK
 501  FWLMGSIKTI LTNCDCACGY LGHNCPITKI KNFSEEFMRN TKESLSNKSY
 551  FNTIMHNCKP
```

FIGURE 41. *YMR134w* DNA Sequence

Sequence contains 1200bp of 5' promoter sequence.
Symbols: 1 to: 1914 from: chr13.gcg ck: 8335, 536637 to: 538550
Chromosome XIII Sequence
Nature 387:90-93 [97313268] (1997) The nucleotide sequence of
Saccharomyces cerevisiae chromosome XIII.
Bowman, S., Churcher, C., Badcock, K., Brown, D., Chillingworth, T.,
Connor, R., Dedman, K., Devlin, K., Gentles, S., Hamlin, N., Hunt, S., . . .

gcgseq.tmp.31828 Length: 1914 March 26, 1999 16:58 Type: N Check: 3324
..

```
   1  TACAATAACA AGCCAGGTGC AAGGCAATAA TAACGGTACA AAGGTCTGTT
  51  TCACAGAAGG TCCAAAAGTT AGTAGCTACA CAAATCCGAA CACGCAATTT
 101  CAAACTCAAA ACATGATTAT GGATTTCAGT CAACGTTATC AGGAAGAATC
 151  TGAAAGAGAG TCAAATAATC GTTCAAATAT AACTTTACCA CACGACAGCA
 201  TTCAAATAGC TCAACAAATA TGGCCAAACA CGGATTTAAA TGTAGTACAA
 251  TCTTCACAAG ACCTCAACAC TCCAATGGCT ACGCAAACTG TTTTGGGTCG
 301  TCCTGAGTCG CTAATTGTAC AGCCATTGGA GGTTTCTCAA TCTCCACCAA
 351  ACACTACCAA CTGCCTTCCT AATGCAGAAA ACAAAAAGAA AAAAGTCGAC
 401  ACCACTTCTG ATTTTACTTC AAGAAAGGAG ATTGCTCTGT GTAAAACTGG
 451  TTTATTAGAA ACTATTCATA TACCAAAGGA AAGGGAAAGT CAGATGCAAA
 501  GCGTCACTGG TTTAGATGCA ACACCAACGA TTATATGGAG CCCCGGGAAA
 551  GACAACACGG CGAAGAAAAA TACCAGTAAT AAGAAAAATA TTGATGATAA
 601  ACTAACAAAC CCCCAAAAAT CTGGAAATAC ACATACCCCT GATAGAAATA
 651  AAGAAGTGCT ACCTAACGGC ACACTTAATG AAACGAGGAA AGAAGCATCG
 701  CCAAGCGAAG GATTAACGAT AAGAGTTAAA AACGTTAATC GGAATGCGTC
 751  AAGAAAAATA TCTAAGCGGC TAATCAAGGA AAAGTTGAAA GACGAAGAAT
 801  TCATGAAATG GGTATGTATG CATTTGCAAG AAACTGAGCT GTTTCCCCCT
 851  CTTATCCACT CATTTTCTCT GACTTGACAA AGAAATACTA ACTAACAACT
 901  TTTGCCACTA CAAATATGAA TGAAAAGGTT AATAAGGTTG AAACGGTTCT
 951  CAATAAAATG TTCGAAAAGT GAACCCTTTT TTTGCAATTC CTTTTTACAC
1001  TAGCCACGAA GTAAAATGGA AAAGTAAACC CGAGTTTCGG CAATATCGCT
1051  AAGCAAGAAG AGCAAGCTCG TTTAAGTAAG CCTTTATGAA AAAAAAACAA
1101  AATATAAAGC ATTATAAAAA TTGAATCACA TCGCAAATCT GCAATATACT
1151  TGGAAGTGTT TATAGCAAAG TGTGGTATAG AAAAAGAACC AAAGGCCGGT
1201  ATGTCGTTAA AGGATAGGTA TCTAAATCTC GAATTAAAAT TAATAAATAA
1251  ACTACAGGAG TTGCCATATG TTCATCAATT TATCCATGAT CGAATAAGTG
1301  GTAGGATAAC TCTCTTTTTG ATAGTGGTTG GTACGCTTGC ATTTTTTAAC
1351  GAACTGTATA TAACGATCGA AATGAGTCTT CTACAAAAGA ACACATCAGA
1401  AGAACTAGAG CGTGGAAGAA TCGATGAAAG TCTGAAGCTT CATCGGATGT
1451  TGGTGAGTGA TGAATATCAC GGTAAAGAAT ACAAAGACGA GAAAAGCGGT
1501  ATTGTTATTG AAGAGTTCGA AGATCGCGAT AAGTTTTTTG CAAAACCTGT
1551  GTTTGTATCA GAATTGGATG TCGAATGTAA TGTTATTGTA GATGGGAAAG
1601  AACTTCTGTC CACCCCATTA AAATTTCATG TTGAATTTTC TCCAGAGGAT
1651  TATGAAAATG AAAAAAGACC TGAGTTTGGT ACTACCTTGC GTGTATTGAG
1701  GCTGAGACTT TACCACTACT TTAAAGATTG CGAAATATAT CGCGATATAA
1751  TTAAGAATGA GGGCGGTGAA GGGGCAAGAA AGTTTACGAT TTCCAACGGT
1801  GTCAAAATTT ACAATCATAA AGATGAACTA CTGCCATTGA ATATCGATGA
1851  TGTTCAATTA TGTTTCCTGA AGATTGATAC GGGAAACACG ATAAAATGCG
1901  AATTCATACT ATGA
```

FIGURE 42. *YMR134w* Protein Sequence

Nature 387:90-93 [97313268] (1997) The nucleotide sequence of Saccharomyces cerevisiae chromosome XIII.

Bowman, S., Churcher, C., Badcock, K., Brown, D., Chillingworth, T., Connor, R., Dedman, K., Devlin, K., Gentles, S., Hamlin, N., Hunt, S., Jagels, K., Lye, G., Moule, S., Odell, C., Pearson, D., Rajandream, et al.

YMR134W Length: 237 March 26, 1999 16:59 Type: P Check: 2966 ..

```
   1  MSLKDRYLNL ELKLINKLQE LPYVHQFIHD RISGRITLFL IVVGTLAFFN

  51  ELYITIEMSL LQKNTSEELE RGRIDESLKL HRMLVSDEYH GKEYKDEKSG

 101  IVIEEFEDRD KFFAKPVFVS ELDVECNVIV DGKELLSTPL KFHVEFSPED

 151  YENEKRPEFG TTLRVLRLRL YHYFKDCEIY RDIIKNEGGE GARKFTISNG

 201  VKIYNHKDEL LPLNIDDVQL CFLKIDTGNT IKCEFIL
```

FIGURE 43. *YER044c* DNA Sequence

Sequence contains 1200bp of 5' promoter sequence.

Symbols: 1 to: 1647 from: chr5.gcg /rev ck: 9036, 237569 to: 239215

Chromosome V Sequence
Nature 387:78-81 [97313264] (1997) The nucleotide sequence of
Saccharomyces cerevisiae chromosome V.
Dietrich, F. S., Mulligan, J., Hennessy, K., Yelton, M. A., Allen, E.,
Araujo, R., Aviles, E., Berno, A., Brennan, T., Carpenter, J., Chen, . . .

gcgseq.tmp.2512 Length: 1647 March 26, 1999 16:38 Type: N Check: 8794 ..

```
   1  AACACTCCAA ATCTTGTTAG TTTCTCATTA TTCGCATCGC ATAGATTCTG
  51  ATTCTTCTTT TAAGAGGACA CTGATAGACG TTCATGTTTT CAATTTCATC
 101  GCCAAGTTTC TGTTTAATAG AATTTTATTG AAGAAGAACC AAAACGATCC
 151  AAAATGGCTT CAAAACTTTT ACGACCAGGG AGATGGCAAA CATTTATGTG
 201  ATAAAGTTGA CTACAAGCGC TTGTGTTCGT TGCATTTTAC CCTTATTTAC
 251  TCTATTATTA ACATTCAACT CATCAAAATC AAGACAAACC AAACATTTGA
 301  ACCGCAGATA TTAAAATACG TATCTGTTCT GAAATTAATT GAACACATAC
 351  TTATCATCAT CGAAAGTCTG ATACATGTAC TTATTAGATT TGTATCGAAG
 401  CATAAACTAA TATGCATCAA CCGGAAAAAG GCGTACTGTC GAGTATACCT
 451  CGAAAGAGAA TTGAGTTTGA AGAAAACCTA CTTAAAGAAC TTTTACAGTG
 501  TAATAAGCGG TGTCCCAGAA AAAGAGTTAG GGGGTCTATT GAAAATACTC
 551  AAGATAGTTA TTCTATCATT GCTCGAGACA TTTGAAAGCA TTGAATGGCA
 601  GCACTTAAAA CCTTTCCTGG AAAAATTTCC GGCTCATGAA ATATCGCTTC
 651  AGAAGAAAAG GAAATATATA CAGGCGGCCT TATTAATTAC TGCCGAAAGA
 701  AATTTGATAG CGCGCTTTCG ATTGTCAAGA TGGTTCAATG AGACAGAAAA
 751  CATTTAATTT TTCTTTTGCA GTAGGAGGCG CATTATAAAA CACAAAAATA
 801  TCGAAAGCTC TTTCATTTCG GGGACAACAA CTTCAGTTGA AAATTACAGT
 851  GAACACAACA TCTTCCCCAA CAGACCTACA TTAAAACGCT TCTTCCGGAC
 901  TTGCCCATGA TTAACCTAAT CTTATACGAA CTGAATTAAA CTTTACGGTA
 951  TTACCGATAG GAAACTTCTA TTTTATGATT TTTTCGTTCG GGGACGGAAC
1001  GAACAGGAAA CAAAAAAAAA GGTACGATCC ATTGTATTCT CTACCCCCGT
1051  ATATAAAACT AAGCTGAACA AGCCTGTTGT TTTGCTTTAC TATTGCTACT
1101  ATTTTTGACG TAAACGCATT GACTAATTTC AGGTTTTTAT ATTCTTGACA
1151  CTAGCTAGAC CATAGTATCG AAGGATTCAA ATACACTAAA GTATCAGATA
1201  ATGTTCAGCC TACAAGACGT AATAACTACA ACCAAGACCA CCTTGGCAGC
1251  AATGCCAAAA GGTTACTTAC CAAAATGGTT ACTTTTCATT TCCATTGTAT
1301  CAGTCTTCAA TTCTATCCAG ACTTACGTTT CTGGTTTAGA ATTGACACGT
1351  AAAGTCTACG AAAGAAAACC CACTGAAACA ACCCATTTGA GTGCAAGAAC
1401  TTTCGGTACT TGGACCTTTA TTTCCTGTGT TATCAGATTC TATGGGGCTA
1451  TGTACTTGAA TGAACCACAC ATTTTCGAAT TGGTCTTCAT GTCTTATATG
1501  GTTGCCCTAT TCCACTTCGG CTCTGAATTA TTGATCTTTA GAACTTGTAA
1551  GTTGGGAAAG GGATTCATGG GTCCATTGGT TGTCTCAACC ACCTCTTTGG
1601  TTTGGATGTA CAAACAAAGA GAATACTACA CTGGTGTTGC TTGGTAA
```

FIGURE 44. *YER044c* Protein Sequence

Nature 387:78-81 [97313264] (1997) The nucleotide sequence of Saccharomyces cerevisiae chromosome V.

Dietrich, F. S., Mulligan, J., Hennessy, K., Yelton, M. A., Allen, E., Araujo, R., Aviles, E., Berno, A., Brennan, T., Carpenter, J., Chen, E., Cherry, J. M., Chung, E., Duncan, M., Guzman, E., Hartzell, G., et al.

YER044C Length: 148 March 26, 1999 16:40 Type: P Check: 3540 ..

1 MFSLQDVITT TKTTLAAMPK GYLPKWLLFI SIVSVFNSIQ TYVSGLELTR

51 KVYERKPTET THLSARTFGT WTFISCVIRF YGAMYLNEPH IFELVFMSYM

101 VALFHFGSEL LIFRTCKLGK GFMGPLVVST TSLVWMYKQR EYYTGVAW

FIGURE 45. Mouse EST with Similarity to *YER044c*

```
LOCUS       AI386195      455 bp    mRNA            EST       27-JAN-1999
DEFINITION  mq60h05.y1 Soares 2NbMT Mus musculus cDNA clone IMAGE:583161 5'
            similar to SW:YEN4_YEAST P40030 HYPOTHETICAL 17.1 KD PROTEIN IN
            SAH1-MEI4 INTERGENIC REGION. ;, mRNA sequence.
ACCESSION   AI386195
NID         g4199658
KEYWORDS    EST.
SOURCE      house mouse.
  ORGANISM  Mus musculus
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Mammalia;
            Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.
REFERENCE   1  (bases 1 to 455)
  AUTHORS   Marra,M., Hillier,L., Kucaba,T., Martin,J., Beck,C., Wylie,T.,
            Underwood,K., Steptoe,M., Theising,B., Allen,M., Bowers,Y.,
            Person,B., Swaller,T., Gibbons,M., Pape,D., Harvey,N., Schurk,R.,
            Ritter,E., Kohn,S., Shin,T., Jackson,Y., Cardenas,M., McCann,R.,
            Waterston,R. and Wilson,R.
  TITLE     The WashU-NCI Mouse EST Project 1999
  JOURNAL   Unpublished (1999)
COMMENT
            Contact: Marra M/WashU-NCI Mouse EST Project 1999
            Washington University School of Medicine
            4444 Forest Park Parkway, Box 8501, St. Louis, MO 63108, USA
            Tel: 314 286 1800
            Fax: 314 286 1810
            Email: mouseest@watson.wustl.edu
            This clone is available royalty-free through LLNL ; contact the
            IMAGE Consortium (info@image.llnl.gov) for further information.
            MGI:357809
            This read is a RESEQUENCE of a previously sequenced mouse clone
            This read has been verified (found to hit its original self in
the
            correct orientation)
            Seq primer: -40RP from Gibco
            High quality sequence stop: 455.
FEATURES              Location/Qualifiers
     source           1..455
                      /organism="Mus musculus"
                      /strain="C57BL/6J"
                      /note="Vector: pT7T3D-Pac (Pharmacia) with a modified
                      polylinker; Site_1: Not I; Site_2: Eco RI; 1st strand
cDNA
                      was primed with a Not I - oligo(dT) primer [5'
                      TGTTACCAATCTGAAGTGGGAGCGGCCGCGTTTTTTTTTTTTTTTTTTTTTTTT
                      3']; double-stranded cDNA was ligated to Eco RI adaptors
                      (Pharmacia), digested with Not I and cloned into the Not
I
                      and Eco RI sites of the modified pT7T3 vector. RNA
                      provided by Dr. Bertrand Jordan. Library went through
two
                      rounds of normalization, and was constructed by Bento
                      Soares and M.Fatima Bonaldo."
                      /db_xref="taxon:10090"
                      /clone="IMAGE:583161"
                      /clone_lib="Soares 2NbMT"
                      /sex="male"
                      /tissue_type="Thymus"
                      /dev_stage="4 weeks"
                      /lab_host="DH10B"
```

```
BASE COUNT       94 a    131 c    112 g    117 t      1 others
ORIGIN
        1 tgcggatgct gctgatactg ctgcagtagt actggatcgt caggcagagc gccctctctt
       61 ggaggggagt catgagccgc ttcctgaatg tgttacgaag ctggctggtt atggtgtcca
      121 ttatagccat ggggaacaca ctccagagct tccgagacca cacttttctc tacgagaagc
      181 tctacactgg caagccaaac cttgtgaatg gcctccaagc ccggaccttt gggatctgga
      241 cgctgctctc atcagtgatt cgctgcctct gtgccattga catccacaac aaaacactct
      301 atcacatcac actgtggaca ttcctcctcg ccctgngaca cttcctctca gagttgtttg
      361 tatttggaac agcagctccc acagttggtg tgctggcacc cctgatggta gcaagtttct
      421 caatcctggg catgctggtc gggctcccgt accta
//
```

FIGURE 45 (cont).

FIGURE 46. Human EST with Similarity to *YER044c*

```
LOCUS       W28235        839 bp    mRNA            EST       08-MAY-1996
DEFINITION  43h8 Human retina cDNA randomly primed sublibrary Homo sapiens
            cDNA, mRNA sequence.
ACCESSION   W28235
NID         g1308183
KEYWORDS    EST.
SOURCE      human.
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Mammalia;
            Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 839)
  AUTHORS   Macke, J., Smallwood, P. and Nathans, J.
  TITLE     Adult Human Retina cDNA
  JOURNAL   Unpublished (1996)
COMMENT
            Contact: Dr. Jeremy Nathans
            Dr. Jeremy Nathans, Dept. of Molecular Biology and Genetics
            Johns Hopkins School of Medicine
            725 North Wolfe Street, Baltimore, MD 21205
            Tel: 410 955 4678
            Fax: 410 614 0827
            Email: jeremy_nathans@qmail.bs.jhu.edu
            Clones from this library are NOT available.
            PCR PRimers
            FORWARD: CTTTTGAGCAAGTTCAGCCTGGTTAAGT
            BACKWARD: GAGGTGGCTTATGAGTATTTCTTCCAGGGTAA
            Seq primer: GGGTAAAAAGCAAAAGAATT.
FEATURES              Location/Qualifiers
     source           1..839
                      /organism="Homo sapiens"
                      /note="Organ: eye; Vector: lambda gt10; Site_1: EcoRI;
                      Site_2: EcoRI; The library used for sequencing was a
                      sublibrary derived from a human retina cDNA library.
                      Inserts from retina cDNA library DNA were isolated,
                      randomly primed, PCR amplified, size-selected, and
cloned
                      into lambda gt10.  Individual plaques were arrayed and
                      used as templates for PCR amplification, and these PCR
                      products were used for sequencing."
                      /db_xref="taxon:9606"
                      /clone_lib="Human retina cDNA randomly primed
sublibrary"
                      /sex="mixed (males and females)"
                      /tissue_type="retina"
                      /dev_stage="adult"
                      /lab_host="E. coli strain K802"
BASE COUNT      127 a    141 c    136 g    140 t    295 others
ORIGIN
        1 gnnnnnngnn nnnnnnnnnt tnttgagnac cgcagtngca gcagcagcag ccgctgncgc
       61 aaacaagccc tcccacgttt gaggggagtc atgagccgtt tcctgaatgt gttaagaagt
      121 tggctggtta tggtgtccat catagccatg gggaacacgc tgcagagctt ccgagaccac
      181 acttttctct atgaaaagct ctacactggc aagccaaacc ttgtgaatgg cctccaagct
      241 cggacctttg ggatctggac gctgctctca tcagtgattc gctgcctctg tgccattgac
      301 attcacaaca agacgctcta tcacatcaca ctctggacct tcctccttgc cctggggcat
      361 ttcctctctg agttgtttgt cttatggaac tgcagctccc acgattggng tcctggcanc
      421 cctgatggtg gnaagtttct ccatcctggg tattgtggtc ggctccngta ttttagaagt
      481 agaaccagtt ccagacagaa gaagagaact gaggcagaat atcaacccca gggtggatca
      541 antgggttac aagtggttna aaannnnnnn nnnnnnnnnc nnnntnntnt naannnnnnn
      601 nnnnnnnnnn nnnnnnnnna nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn
```

```
      661 nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn
      721 nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn
      781 nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnc
//
```

FIGURE 46 (cont).

FIGURE 47. Rat EST with Similarity to *YER044c*

```
LOCUS       AI172515      475 bp    mRNA            EST       11-FEB-1999
DEFINITION  UI-R-C2p-nu-d-02-0-UI.s1 UI-R-C2p Rattus norvegicus cDNA clone
            UI-R-C2p-nu-d-02-0-UI 3', mRNA sequence.
ACCESSION   AI172515
NID         g3712555
KEYWORDS    EST.
SOURCE      Norway rat.
  ORGANISM  Rattus norvegicus
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Mammalia;
            Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Rattus.
REFERENCE   1  (bases 1 to 475)
  AUTHORS   Bonaldo,M.F., Lennon,G. and Soares,M.B.
  TITLE     Normalization and subtraction: two approaches to facilitate gene
            discovery
  JOURNAL   Genome Res. 6 (9), 791-806 (1996)
  MEDLINE   97044477
COMMENT
            Contact: Soares, MB
            Program for Rat Gene Discovery and Mapping
            University of Iowa
            451 Eckstein Medical Research Building Iowa City, IA 52242, USA
            Tel: 319 335 8250
            Fax: 319 335 9565
            Email: msoares@blue.weeg.uiowa.edu
            The sequence tag present in the cDNA between the NotI site and
the
            oligo-dT track served to identify it as a clone from the
normalized
            adult Placenta library. cDNA Library Preparation: M. Fatima
            Bonaldo, Ph.D. Clone distribution: clones will be available
through
            Research Genetics
            Seq primer: M13 Forward.
FEATURES              Location/Qualifiers
     source           1..475
                      /organism="Rattus norvegicus"
                      /strain="Sprague-Dawley"
                      /note="Vector: pT7T3D-Pac (Pharmacia) with a modified
                      polylinker; Site_1: Not I; Site_2: Eco RI; The UI-R-C2p
                      library is a subtracted library derived from the UI-R-C1
                      library, which is a subtracted library derived from the
                      UI-R-C0 library.  The UI-R-C0 library consisted of a
                      mixture of individually tagged normalized  libraries
                      constructed from rat placenta, adult lung, brain, liver,
                      kidney,  heart, spleen, ovary, muscle, 8, 12 and 18-day
                      embryo. The tag is a string of  3-5 nucleotides present
                      between the Not I site and the oligo-dT track which
allows
                      identification of the library of origin of a clone
within
                      the mixture. The subtracted library (UI-R-C2p) was
                      constructed as follows: PCR amplified cDNA inserts from
                      UI-R-C1 clones from which 3' ESTs had been derived was
                      used as a driver in a hybridization with the UI-R-C1
                      library in the form of single-stranded circles. The
                      remaining single-stranded circles (subtracted library)
was
                      purified by hydroxyapatite column chromatography,
                      converted to double-stranded circles and electroporated
```

```
                    into DH10B bacteria (Life Technologies) to generate the
                    UI-R-C2p library. This procedure has been previously
                    described (Bonaldo, Lennon and Soares, Genome Research
6:
                    791-806, 1996)"
                    /db_xref="taxon:10116"
                    /clone="UI-R-C2p-nu-d-02-0-UI"
                    /clone_lib="UI-R-C2p"
                    /dev_stage="adult"
                    /lab_host="DH10B (Life Technologies)"
BASE COUNT      115 a    112 c    126 g    119 t      3 others
ORIGIN
        1 ttttttttct ttttttctg tctggatact ggttctgctt ctaggtaccg gagcccaact
       61 agcataccca ggattgagaa acttgctacc atcaagggtg ccagcacacc aactgtggga
      121 gccgctgttc caaatacaaa caactccgag aggaagtgtc ccagggcaag gaggaatgtc
      181 cacagtgtga tgtgatagag tgttttgttg tggatgtcaa tggcacagag gcagcgaatc
      241 actgaagaga gcagcgtcca gatcccaaag gtccgggctt ggaggccatt cacaaggttt
      301 ggtttgccag tgtanagctt ttcatanaga aaagtgtggt ctcggaagct ctggagcgtg
      361 ttncccatgg ctatgatgga caccataacc agccagcttc gtagcacatt caggaagcgg
      421 ctcatgactc ccctcaaaga gagggcgctc tgcctgaccc tcgtgccgaa ttctt
//
```

FIGURE 47 (cont)

FIGURE 48. YLR100w DNA Sequence

Sequence contains 800bp of 5' promoter sequence.
Symbols: 1 to: 1844 from: chr12.gcg ck: 2436, 341011 to: 342854
Chromosome XII Sequence
Nature 387:87-90 [97313267] (1997) The nucleotide sequence of
Saccharomyces cerevisiae chromosome XII.
Johnston, M., Hillier, L., Riles, L., Albermann, K., Andre, B.,
Ansorge, W., Benes, V., Bruckner, M., Delius, H., Dubois, E., . . .

gcgseq.tmp.10136 Length: 1844 March 26, 1999 15:19 Type: N Check: 2071
..

```
   1  ACGTACAAAA AAGAGCACGC TGCTTTATTT ATACTTTTGT GCCACAAGAA
  51  TGATCAACAT CAACATAAAT ATCAACTAGT ATCTGCAACA CATCTGCTCC
 101  ACGGAACTAA ACCCGTTGAG CAGTGCCCCG TGGAAACGTA AACTATCGCA
 151  AATTGGGATT AACAAGCCAA AAACAGCCAA GCAAGATTCA CGAAACCGCG
 201  CCTCGTTTGG ACCCCGAAGG CCCATTTAAC GGCCGGCCGT TACAAGCAAG
 251  ATCGGCAGAG CAAACCACTC CCCAGCACCA CAGCACATCA CTGCACGAGC
 301  AACAATAACT AGAACATGGC AGATAGCGAG GATACCTCTG TGATCCTGCA
 351  GGGCATCGAC ACAATCAACA GCGTGGAGGG CCTGGAAGAA GATGGTTACC
 401  TCAGCGACGA GGACACGTCA CTCAGCAACG AGCTCGCAGA TGCACAGCGT
 451  CAATGGGAAG AGTCGCTGCA ACAGTTGAAC AAGCTGCTCA ACTGGGTCCT
 501  GCTGCCCCTG CTGGGCAAGT ATATAGGTAG GAGAATGGCC AAGACTCTAT
 551  GGAGTAGGTT CATTGAACAC TTTGTATAAG TGTTTGTTGT TTATGTATCC
 601  GCATATAGCA GTTATAACAG ATAAATGGCA CTTTTCGCAC ACCCGTTGTT
 651  TTATCTCCGA TAGTACGTGG GCCTTTATTT ATGGTCGTTT AACGAAAGAA
 701  CGGCATCTTG AATTGAGCAG GTATTTAAAA GATAGGACGA GAAACAAGCA
 751  CATGATCTGT GTCGAAAAAA AGTAGCAAAG AGAAAAAGTA GGAGGATAGG
 801  ATGAACAGGA AAGTAGCTAT CGTAACGGGT ACTAATAGTA ATCTTGGTCT
 851  GAACATTGTG TTCCGTCTGA TTGAAACTGA GGACACCAAT GTCAGATTGA
 901  CCATTGTGGT GACTTCTAGA ACGCTTCCTC GAGTGCAGGA GGTGATTAAC
 951  CAGATTAAAG ATTTTTACAA CAAATCAGGC CGTGTAGAGG ATTTGGAAAT
1001  AGACTTTGAT TATCTGTTGG TGGACTTCAC CAACATGGTG AGTGTCTTGA
1051  ACGCATATTA CGACATCAAC AAAAAGTACA GGGCGATAAA CTACCTTTTC
1101  GTGAATGCTG CGCAAGGTAT CTTTGACGGT ATAGATTGGA TCGGAGCGGT
1151  CAAGGAGGTT TTCACCAATC CATTGGAGGC AGTGACAAAT CCGACATACA
1201  AGATACAACT GGTGGGCGTC AAGTCTAAAG ATGACATGGG GCTTATTTTC
1251  CAGGCCAATG TGTTTGGTCC GTACTACTTT ATCAGTAAAA TTCTGCCTCA
1301  ATTGACCAGG GGAAAGGCTT ATATTGTTTG GATTTCGAGT ATTATGTCCG
1351  ATCCTAAGTA TCTTTCGTTG AACGATATTG AACTACTAAA GACAAATGCC
1401  TCTTATGAGG GCTCCAAGCG TTTAGTTGAT TTACTGCATT TGGCCACCTA
1451  CAAAGACTTG AAAAAGCTGG GCATAAATCA GTATGTAGTT CAACCGGGCA
1501  TATTTACAAG CCATTCCTTC TCCGAATATT TGAATTTTTT CACCTATTTC
1551  GGCATGCTAT GCTTGTTCTA TTTGGCCAGG CTGTTGGGGT CTCCATGGCA
1601  CAATATTGAT GGTTATAAAG CTGCCAATGC CCCAGTATAC GTAACTAGAT
1651  TGGCCAATCC AAACTTTGAG AAACAAGACG TAAAATACGG TTCTGCTACC
1701  TCTAGGGATG GTATGCCATA TATCAAGACG CAGGAAATAG ACCCTACTGG
1751  AATGTCTGAT GTCTTCGCTT ATATACAGAA GAAGAAACTG GAATGGGACG
1801  AGAAACTGAA AGATCAAATT GTTGAAACTA GAACCCCCAT TTAA
```

FIGURE 49. *YLR100w* Protein Sequence

Nature 387:87-90 [97313267] (1997) The nucleotide sequence of Saccharomyces cerevisiae chromosome XII.

Johnston, M., Hillier, L., Riles, L., Albermann, K., Andre, B., Ansorge, W., Benes, V., Bruckner, M., Delius, H., Dubois, E., Dusterhoft, A., Entian, K. D., Floeth, M., Goffeau, A., Hebling, U., et al.

YLR100W Length: 347 March 26, 1999 15:20 Type: P Check: 2853 ..

```
   1  MNRKVAIVTG TNSNLGLNIV FRLIETEDTN VRLTIVVTSR TLPRVQEVIN

  51  QIKDFYNKSG RVEDLEIDFD YLLVDFTNMV SVLNAYYDIN KKYRAINYLF

 101  VNAAQGIFDG IDWIGAVKEV FTNPLEAVTN PTYKIQLVGV KSKDDMGLIF

 151  QANVFGPYYF ISKILPQLTR GKAYIVWISS IMSDPKYLSL NDIELLKTNA

 201  SYEGSKRLVD LLHLATYKDL KKLGINQYVV QPGIFTSHSF SEYLNFFTYF

 251  GMLCLFYLAR LLGSPWHNID GYKAANAPVY VTRLANPNFE KQDVKYGSAT

 301  SRDGMPYIKT QEIDPTGMSD VFAYIQKKKL EWDEKLKDQI VETRTPI
```

FIGURE 50. Human EST with Similarity to *YLR100w*

```
LOCUS       R92053        454 bp    mRNA            EST      25-AUG-1995
DEFINITION  yp96c01.r1 Soares fetal liver spleen 1NFLS Homo sapiens cDNA
clone
            IMAGE:195264 5', mRNA sequence.
ACCESSION   R92053
NID         g959593
KEYWORDS    EST.
SOURCE      human.
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Mammalia;
            Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 454)
  AUTHORS   Hillier,L., Clark,N., Dubuque,T., Elliston,K., Hawkins,M.,
            Holman,M., Hultman,M., Kucaba,T., Le,M., Lennon,G., Marra,M.,
            Parsons,J., Rifkin,L., Rohlfing,T., Soares,M., Tan,F.,
            Trevaskis,E., Waterston,R., Williamson,A., Wohldmann,P. and
            Wilson,R.
  TITLE     The WashU-Merck EST Project
  JOURNAL   Unpublished (1995)
COMMENT
            Contact: Wilson RK
            Washington University School of Medicine
            4444 Forest Park Parkway, Box 8501, St. Louis, MO 63108
            Tel: 314 286 1800
            Fax: 314 286 1810
            Email: est@watson.wustl.edu
            Insert Size: 1067
            High quality sequence stops: 337
            Source: IMAGE Consortium, LLNL
            This clone is available royalty-free through LLNL ; contact the
            IMAGE Consortium (info@image.llnl.gov) for further information.
            Insert Length: 1067   Std Error: 0.00
            Seq primer: M13RP1
            High quality sequence stop: 337.
FEATURES             Location/Qualifiers
     source          1..454
                     /organism="Homo sapiens"
                     /note="Organ: Liver and Spleen; Vector: pT7T3D
(Pharmacia)
                     with a modified polylinker; Site_1: Pac I; Site_2: Eco
RI;
                     1st strand cDNA was primed with a Pac I - oligo(dT)
primer
                     [5' AACTGGAAGAATTAATTAAAGATCTTTTTTTTTTTTTTTTTT 3'],
                     double-stranded cDNA was ligated to Eco RI adaptors
                     (Pharmacia), digested with Pac I and cloned into the Pac
I
                     and Eco RI sites of the modified pT7T3 vector.  Library
                     went through one round of normalization. Library
                     constructed by Bento Soares and M.Fatima Bonaldo."
                     /db_xref="GDB:3764314"
                     /db_xref="taxon:9606"
                     /clone="IMAGE:195264"
                     /clone_lib="Soares fetal liver spleen 1NFLS"
                     /sex="male"
                     /dev_stage="20 week-post conception fetus"
                     /lab_host="DH10B (ampicillin resistant)"
BASE COUNT      115 a    111 c     96 g    129 t      3 others
```

```
ORIGIN
        1 tttgagacca atgtctttgg ccattttatc ctgattcggg aactggagcc tctcctctgt
       61 cacagtgaca atccatctca gctcatctgg acatcatctc gcagtgcaag gaaatctaat
      121 ttcagcctcg aggacttcca gcacagcaaa ggcaaggaac cctacagctc ttccaaatat
      181 gccactgacc ttttgagtgt ggctttgaac aggaacttca accagcaggg tctctattcc
      241 aatgtggcct gtccaggtac agcattgacc aatttgacat atggaattct gcctccgttt
      301 atatggacgc tgttggatgc cggcaatatt gctacttcgc ttttttggca aatggcattc
      361 actttggaca ccatataatg ggaacaggaa gntatgggta tgggnttttc ccaccaaaag
      421 gctggaatcn tttcaatcct ctggatccaa atat
//
```

FIGURE 50 (cont).

FIGURE 51. Mouse EST with Similarity to *YLR100w*

```
LOCUS       AI226514     1039 bp    mRNA            EST       29-OCT-1998
DEFINITION  uj07d08.y1 Sugano mouse liver mlia Mus musculus cDNA clone
            IMAGE:1891215 5' similar to TR:Q62904 Q62904 OVARIAN-SPECIFIC
            PROTEIN. ;, mRNA sequence.
ACCESSION   AI226514
NID         g3809567
KEYWORDS    EST.
SOURCE      house mouse.
  ORGANISM  Mus musculus
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Mammalia;
            Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.
REFERENCE   1  (bases 1 to 1039)
  AUTHORS   Marra,M., Hillier,L., Allen,M., Bowles,M., Dietrich,N.,
Dubuque,T.,
            Geisel,S., Kucaba,T., Lacy,M., Le,M., Martin,J., Morris,M.,
            Schellenberg,K., Steptoe,M., Tan,F., Underwood,K., Moore,B.,
            Theising,B., Wylie,T., Lennon,G., Soares,B., Wilson,R. and
            Waterston,R.
  TITLE     The WashU-HHMI Mouse EST Project
  JOURNAL   Unpublished (1996)
COMMENT
            Contact: Marra M/Mouse EST Project
            WashU-HHMI Mouse EST Project
            Washington University School of MedicineP
            4444 Forest Park Parkway, Box 8501, St. Louis, MO 63108
            Tel: 314 286 1800
            Fax: 314 286 1810
            Email: mouseest@watson.wustl.edu
            This clone is available royalty-free through LLNL ; contact the
            IMAGE Consortium (info@image.llnl.gov) for further information.
            MGI:975539
            Seq primer: custom primer used
            High quality sequence stop: 509.
FEATURES             Location/Qualifiers
     source          1..1039
                     /organism="Mus musculus"
                     /strain="C57BL"
                     /note="Organ: liver; Vector: pME18S-FL3; Site_1: DraIII
                     (CACTGTGTG); Site_2: DraIII (CACCATGTG); 1st strand cDNA
                     was primed with an oligo(dT) primer
                     [ATGTGGCCTTTTTTTTTTTTTTTT]; double-stranded cDNA was
                     ligated to a  DraIII adaptor [TGTTGGCCTACTGG], digested
                     and cloned into distinct DraIII sites of the pME18S-FL3
                     vector (5' site CACTGTGTG, 3' site CACCATGTG). XhoI
should
                     be used to isolate the cDNA insert.  Size selection was
                     performed to exclude fragments <1.5kb.  Library
                     constructed by Dr. Sumio Sugano (University of Tokyo
                     Institute of Medical Science).  Custom primers  for
                     sequencing: 5' end primer CTTCTGCTCTAAAAGCTGCG and 3'
end
                     primer CGACCTGCAGCTCGAGCACA."
                     /db_xref="taxon:10090"
                     /clone="IMAGE:1891215"
                     /clone_lib="Sugano mouse liver mlia"
                     /sex="female"
                     /dev_stage="adult"
                     /lab_host="DH10B"
```

```
BASE COUNT      245 a    267 c    251 g    272 t      4 others
ORIGIN
        1 ggctaagaga accccggtgc agttctactt cggtgcaggg cgtggaagat gcggaaggtg
       61 gttttgatca ccggggcgag cagtggcatt gggctagccc tttgcggtcg actgctggca
      121 gaagacgatg acctccacct gtgtttggcg tgtaggaacc tgagcaaagc aagagctgtt
      181 cgagataccc tgctggcctc tcacccctcc gccgaagtca gcatcgtgca gatggatgtc
      241 agcagcctgc agtcggtggt ccggggtgca gaggaagtca agcaaaagtt tcaaagatta
      301 gactacttat atctgaatgc tggaatcctg cctaatccac aattcaacct caaggcattt
      361 ttctgcggca tcttttcaag aaatgtgatt catatgttca ccacagcgga aggaattttg
      421 acccagaatg actcggtcac tgccgacggg ttgcaggagg tgtttgaaac caatctcttt
      481 ggccacttta ttctgattcg ggaactggaa ccacttctct gccatgccga caacccctct
      541 cagctcatct ggacgtcctc tcgcaatgca aagaaggcta acttcagcct ggaggacata
      601 cagcacttca aaggcccgga accctacagc tctttccaat atgctaccga cctcctgaat
      661 gtggctntga acagggaatt caaaccagaa ggtctggtat tcagtggtga ttgccgaggg
      721 cgtctgatga ccaatatgac gtatggaaat ttgccttcct ttatcctgac cgtggttcta
      781 cccttaagtg ggctccttcg ctttttttgaa aatgccctca cctgggaccc cgtaccactg
      841 atcaaaagct ctgggtgtgt ttctttcaca tataaccgga ggcttttatt ctttgaccaa
      901 atacgcgagc tccaccttgg tagtgggact atataccgac cggtcccacg aatgcactca
      961 tttaacacct tgtcaaaact ttttatagtt ttacctgttg tgataacgtg gtgntacccc
     1021 cttcgtantt gnaataccc
//
```

FIGURE 51 (cont).

FIGURE 52. Mouse EST with Similarity to *YLR100w*

```
LOCUS       AI528381      837 bp    mRNA            EST       18-MAR-1999
DEFINITION  ui96g06.y1 Sugano mouse liver mlia Mus musculus cDNA clone
            IMAGE:1890298 5' similar to TR:Q62904 Q62904 OVARIAN-SPECIFIC
            PROTEIN. ;, mRNA sequence.
ACCESSION   AI528381
NID         g4442516
KEYWORDS    EST.
SOURCE      house mouse.
  ORGANISM  Mus musculus
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Mammalia;
            Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.
REFERENCE   1  (bases 1 to 837)
  AUTHORS   Marra,M., Hillier,L., Kucaba,T., Martin,J., Beck,C., Wylie,T.,
            Underwood,K., Steptoe,M., Theising,B., Allen,M., Bowers,Y.,
            Person,B., Swaller,T., Gibbons,M., Pape,D., Harvey,N., Schurk,R.,
            Ritter,E., Kohn,S., Shin,T., Jackson,Y., Cardenas,M., McCann,R.,
            Waterston,R. and Wilson,R.
  TITLE     The WashU-NCI Mouse EST Project 1999
  JOURNAL   Unpublished (1999)
COMMENT
            Contact: Marra M/WashU-NCI Mouse EST Project 1999
            Washington University School of Medicine
            4444 Forest Park Parkway, Box 8501, St. Louis, MO 63108, USA
            Tel: 314 286 1800
            Fax: 314 286 1810
            Email: mouseest@watson.wustl.edu
            This clone is available royalty-free through LLNL ; contact the
            IMAGE Consortium (info@image.llnl.gov) for further information.
            MGI:974622
            Possible reversed clone: similarity on wrong strand
            Seq primer: custom primer used
            High quality sequence stop: 429.
FEATURES              Location/Qualifiers
     source           1..837
                      /organism="Mus musculus"
                      /strain="C57BL"
                      /note="Organ: liver; Vector: pME18S-FL3; Site_1: DraIII
                      (CACTGTGTG); Site_2: DraIII (CACCATGTG); 1st strand cDNA
                      was primed with an oligo(dT) primer
                      [ATGTGGCCTTTTTTTTTTTTTTTT]; double-stranded cDNA was
                      ligated to a  DraIII adaptor [TGTTGGCCTACTGG], digested
                      and cloned into distinct DraIII sites of the pME18S-FL3
                      vector (5' site CACTGTGTG, 3' site CACCATGTG). XhoI
should
                      be used to isolate the cDNA insert.  Size selection was
                      performed to exclude fragments <1.5kb.  Library
                      constructed by Dr. Sumio Sugano (University of Tokyo
                      Institute of Medical Science).  Custom primers  for
                      sequencing: 5' end primer CTTCTGCTCTAAAAGCTGCG and 3'
end
                      primer CGACCTGCAGCTCGAGCACA."
                      /db_xref="taxon:10090"
                      /clone="IMAGE:1890298"
                      /clone_lib="Sugano mouse liver mlia"
                      /sex="female"
                      /dev_stage="adult"
                      /lab_host="DH10B"
```

```
BASE COUNT      191 a    222 c    212 g    208 t      4 others
ORIGIN
        1 ggctaagaga accccggtgc agttctactt cggtgcaggg cgtggaagat gcggaaggtg
       61 gttttgatca ccggggcgag cagtggcatt gggctagccc tttgcggtcg actgctggca
      121 gaagacgatg acctccacct gtgtttggcg tgtaggaacc tgagcaaagc aagagctgtt
      181 cgagataccc tgctggcctc tcacccctcc gccgaagtca gcatcgtgca gatggatgtc
      241 agcagcctgc agtcggtggt ccggggtgca gaggaagtca agcaaaagtt tcaaagatta
      301 gactacttat atctgaatgc tggaatcctg cctaatccac aattcaacct caaggcattt
      361 ttctgcggca tcttttcaag aaatgtgatt catatgttca ccacagcgga aggaattttg
      421 acccagaatg actcggtcac tgccgaccgg ttgcaggagg tgtttgaaac caatctctct
      481 tgccacttta ttctgattcg ggaactggaa ccacttctct tgcatgcgga caacccctct
      541 cagctcatct ggacgtcctc tcgcaatgca nagaaggcta acttcagcct ggaggacatn
      601 cagcactcca tagggcccgg accctacagc tctttccaat atgctaccga cctcctgaat
      661 gtggctttga acangaatnt caaccagaag ggtctgtatt ccagtcgcat gtgcccaggc
      721 gtcgtgatga ccaatatgac gtatggaatc ttgcctccct tttatctgga cgtgctccta
      781 cccatgatgg tgctccttcg cttctttggt aatgcgctta ctgggacacc gtacaac
//
```

FIGURE 52 (cont).

FIGURE 53. Mouse Gene with Similarity to *YLR100w*

```
LOCUS       3319971      334 aa                                  14-JUL-1998
DEFINITION  17-beta-hydroxysteroid dehydrogenase type 7.
ACCESSION   3319971
PID         g3319971
DBSOURCE    EMBL: locus MMY15733, accession Y15733
KEYWORDS    .
SOURCE      house mouse.
  ORGANISM  Mus musculus
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Rodentia; Sciurognathi; Muridae; Murinae; Mus.
REFERENCE   1  (residues 1 to 334)
  AUTHORS   Nokelainen,P., Peltoketo,H., Vihko,R. and Vihko,P.
  TITLE     Expression cloning of a novel estrogenic mouse 17
            beta-hydroxysteroid dehydrogenase/17-ketosteroid reductase
            (m17HSD7), previously described as a prolactin receptor-
            associated protein (PRAP) in rat
  JOURNAL   Mol. Endocrinol. 12 (7), 1048-1059 (1998)
  MEDLINE   98322544
REFERENCE   2  (residues 1 to 334)
  AUTHORS   Nokelainen,P.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (27-NOV-1997) P.A. Nokelainen, University of Oulu,
            Biocenter Oulu, WHO Collaborating Centre for Research on
            Reproductive Health Department of Clinical Chemistry, Kajaanintie
            50, FIN-90220 Oulu, FINLAND
FEATURES             Location/Qualifiers
     source          1..334
                     /organism="Mus musculus"
                     /strain="BALB/c"
                     /db_xref="taxon:10090"
                     /tissue_type="mammary gland"
                     /cell_type="epithelial cell derived from mammary gland
of
                     a pregnant mouse"
                     /clone_lib="cDNA library prepared from poly(A)-enriched
                     RNA isolated from HC11 cell line"
                     /clone="m17HSD7.1"
                     /clone="m17HSD7.2"
     Protein         1..334
                     /product="17-beta-hydroxysteroid dehydrogenase type 7"
     CDS             1..334
                     /gene="HSD17B7"
                     /db_xref="SPTREMBL:O88736"
                     /coded_by="Y15733:64..1068"
ORIGIN
        1 mrkvvlitga ssgiglalcg rllaedddlh lclacrnlsk aravrdtlla shpsaevsiv
       61 qmdvsslqsv vrgaeevkqk fqrldylyln agilpnpqfn lkaffcgifs rnvihmftta
      121 egiltqndsv tadglqevfe tnlfghfili relepllcha dnpsqliwts srnakkanfs
      181 lediqhskgp epysssskyat dllnvalnrn fnqkglyssv mcpgvvmtnm tygilppfiw
      241 tlllpimwll rffvnaltvt pyngaealvw lfhqkpesln pltkyasats gfgtnyvtgq
      301 kmdidedtae kfyevllele krvrttvqks dhps
//
```

FIGURE 54. *YER034w* DNA Sequence

Sequence contains 559bp of 5' promoter sequence.
Symbols: 1 to: 1117 from: chr5.gcg ck: 9036, 221286 to: 222402
Chromosome V Sequence
Nature 387:78-81 [97313264] (1997) The nucleotide sequence of
Saccharomyces cerevisiae chromosome V.
Dietrich, F. S., Mulligan, J., Hennessy, K., Yelton, M. A., Allen, E.,
Araujo, R., Aviles, E., Berno, A., Brennan, T., Carpenter, J., Chen, . . .

gcgseq.tmp.6597 Length: 1117 March 26, 1999 16:54 Type: N Check: 5026 ..

```
   1  TGATGAAATA TTCCAGTTAT GCGTGTGCGT CTTGTGATGC AGATCCTTTT
  51  GGGCAAAAAC AGTTGGTTTG TGCGAAAACG CAAGGTAATA AATAGGCTTA
 101  AAGGAACTAA AAAAAAAAAA AGGAAAATAA CCAGCTAAGA TTTAAGGTAC
 151  AAGAAAGCGG TTGCACCTCA AGTAATGATA GTTATTAAAC CTTGGATTGG
 201  ACCAGATGTT TAAAATTGTT TTCAATAGTA GATTTGCAGT CGTAAATGCG
 251  TTCTCAGCAA TATCATATTG TGTTTATGAA GTATTACCAA ACGGGTAGAA
 301  GAACGGTTTA AGAGAATATG TCCGGATAAA GCGATCAGGA GAAAAGCTTA
 351  AAACCCAAAG TGGTCAATCT GCAGCCCATT TAGGCACTCT GCATTTAACC
 401  GATACCCGGA TTGAAGAAAG CTGGCGGGTG TATGGGTGAA GGAGAAGAAA
 451  GGAAGTGATT AGGAGAAACC TCATGGAGAT GAGCACATGC TACAACTAAT
 501  AACGTTATTC TACTTAAAAC GAGCAAAACA AAAAAAAAAA CAAGACAATT
 551  GAAAACGCAA TGGATGCATT CAGCTTAAAG AAGGATAATC GAAAAAAATT
 601  TCAAGATAAA CAGAAATTGA AAAGAAAACA TGCCACACCC AGTGATAGAA
 651  AGTACCGGCT ATTGAACCGC CAAAAAGAAG AGAAAGCTAC CACAGAGGAG
 701  AAAGATCAAG ACCAAGAACA GCCCGCCCTG AAGTCAAACG AGGACAGGTA
 751  CTATGAGGAC CCGGTACTCG AGGACCCGCA TTCTGCAGTC GCCAATGCAG
 801  AGTTGAACAA GGTGCTAAAA GACGTCCTCA AAAATCGGCT CCAGCAGAAC
 851  GACGACGCCA CAGCCGTCAA TAATGTTGCT AATAAAGATA CTTTGAAAAT
 901  CAAAGACCTC AAGCAGATGA ATACGGATGA GCTCAATCGT TGGCTCGGAC
 951  GGCAGAATAC AACATCGGCT ATAACAGCGG CTGAGCCCGA ATCATTAGTC
1001  GTTCCCATTC ACGTACAAGG TGATCATGAT CGTGCGGGCA AGAAGATCAG
1051  TGCCCCTTCG ACCGATCTAC CGGAAGAACT AGAGACCGAT CAGGATTTCC
1101  TTGATGGACT GCTCTAA
```

FIGURE 55. *YER034w* Protein Sequence

Nature 387:78-81 [97313264] (1997) The nucleotide sequence of Saccharomyces cerevisiae chromosome V.

Dietrich, F. S., Mulligan, J., Hennessy, K., Yelton, M. A., Allen, E., Araujo, R., Aviles, E., Berno, A., Brennan, T., Carpenter, J., Chen, E., Cherry, J. M., Chung, E., Duncan, M., Guzman, E., Hartzell, G., et al.

YER034W Length: 185 March 26, 1999 16:55 Type: P Check: 3501 ..

```
   1  MDAFSLKKDN RKKFQDKQKL KRKHATPSDR KYRLLNRQKE EKATTEEKDQ

  51  DQEQPALKSN EDRYYEDPVL EDPHSAVANA ELNKVLKDVL KNRLQQNDDA

 101  TAVNNVANKD TLKIKDLKQM NTDELNRWLG RQNTTSAITA AEPESLVVPI

 151  HVQGDHDRAG KKISAPSTDL PEELETDQDF LDGLL
```

FIGURE 56. *YKL077w* DNA Sequence

Sequence contains 1200bp of 5' promoter sequence.
Symbols: 1 to: 2379 from: chr11.gcg ck: 9298, 289895 to: 292273
Chromosome XI Sequence
Nature 387:98-102 [97313270] (1997) The nucleotide sequence of
Saccharomyces cerevisiae chromosome XV.
Dujon, B., Albermann, K., Aldea, M., Alexandraki, D., Ansorge, W.,
Arino, J., Benes, V., Bohn, C., Bolotin-Fukuhara, M., Bordonne, R., . . .

gcgseq.tmp.4920 Length: 2379 March 26, 1999 16:48 Type: N Check: 4118 ..

```
   1  GAAAGGAAGC TATAGTAATG GGGCTTCAGG AACTTTATGA ATTGGGTGCT
  51  CTTGACACTC GTGGAAAGAT AACTAAACGG GGTCAACAAA TGGCTCTGTT
 101  ACCGCTACAA CCGCATTTAA GTAGTGTCTT AATTAAAGCC AGTGAAGTCG
 151  GATGTTTGAG TCAGGTCATT GATATCGTCT CTTGCCTTAG TGTGGAAAAT
 201  TTACTGTTGA ATCCGTCACC AGAAGAAAGA GATGAGGTGA ACGAGCGTCG
 251  TTTGTCCTTA TGCAACGCTG GTAAAAGGTA TGGTGACCTT ATCATGCTGA
 301  AAGAGCTTTT TGATATCTAT TTCTACGAAC TAGGGAAAAG TCAAGATGCA
 351  AGCTCTGAAA GAAATGATTG GTGTAAAGGA TTGTGTATTT CGATACGTGG
 401  GTTTAAAAAT GTAATTCGTG TCAGAGACCA GTTAAGAGTT TATTGTAAGC
 451  GTTTGTTTTC TTCAATCAGT GAAGAGGATG AAGAATCCAA AAAGATTGGT
 501  GAAGATGGCG AGCTAATTTC GAAAATTTTA AAGTGTTTCT TAACTGGGTT
 551  TATCAAGAAT ACAGCTATAG GGATGCCAGA CAGGTCTTAT AGAACTGTTT
 601  CCACTGGAGA GCCGATAAGC ATTCATCCAT CATCTATGCT ATTTATGAAT
 651  AAAAGCTGCC CCGGTATAAT GTACACGGAG TATGTCTTTA CTACGAAGGG
 701  ATATGCCAGA AATGTTAGTA GGATTGAACT TTCATGGTTA CAAGAAGTTG
 751  TCACTAATGC AGCCGCTGTA GCAAAGCAAA AAGTTTCTGA TTCAAAATAA
 801  GTCACCTACT CTTAGCGCAT TTTTATTGTA TATAAAGGCA TTTAATGTAA
 851  TTTATAGAGC ATTATAAATC GTAACAACTA CTGCAGTATG AGTTTCATGG
 901  ATTCATTTCT CAATATCTTA TGAATATACA CAGGTATATA TGTATATTCA
 951  TGTTAAACGC CTTTCGAATT GTTCGTTGGC TTTTTTTGTG AAATTATCTC
1001  GGGAAAAGGG CGAAATTATA TTATTTTGCC GTTGACATTT TGAAAAGGAA
1051  TAAAAGATCA TGAAAAAAAT AAGAAAGGCA ATTCGACGCA TTTCTCTCAG
1101  CAAGCTATTC TTTACTTTTG AAGAACAAAA TATTTTAGCA AAAAGGTTAA
1151  GACAATATAG TCGGAAGCAG TTCTGCGGGA TCTGAAGGAA TTGCGGAATA
1201  ATGAGATTTC ACGATAGTAT ACTTATCTTC TTTTCTTTGG CATCGCTTTA
1251  TCAACATGTT CATGGTGCAA GACAAGTCGT TCGTCCAAAG GAGAAAATGA
1301  CTACTTCAGA AGAAGTTAAA CCTTGGTTAC GTACGGTTTA TGGAAGTCAA
1351  AAAGAATTAG TCACTCCTAC GGTCATTGCC GGTGTCACTT TTTCTGAAAA
1401  ACCAGAAGAA ACACCAAATC CATTGAAACC TTGGGTATCT TTAGAGCATG
1451  ATGGTAGGCC AAAAACCATT AAACCAGAAA TCAACAAAGG TCGAACCAAG
1501  AAGGGAAGAC CTGATTACTC AACTTACTTC AAAACGGTAA GTTCCCACAC
1551  ATATTCTTAT GAAGAATTGA AGGCTCACAA TATGGGCCCT AATGAAGTTT
1601  TTGTAGAAGA AGAGTATATT GATGAAGATG ACACCTACGT CTCCCTGAAT
1651  CCTATTGTCA GATGTACTCC TAATCTTTAC TTCAATAAAG GTCTAGCAAA
1701  GGATATCCGC AGTGAGCCAT TTTGTACCCC TTATGAGAAT TCTAGATGGA
1751  AGGTTGACAA GACTTACTTC GTTACTTGGT ATACAAGATT TTTTACAGAT
1801  GAGAATTCCG GTAAAGTTGC TGATAAGGTT CGTGTTCATT TGTCCTATGT
1851  TAAAGAAAAC CCCGTAGAGA AGGGCAATTA TAAAAGAGAT ATCCCTGCAA
1901  CTTTTTTCTC TTCCGAATGG ATTGATAATG ACAACGGTCT AATGCCGGTT
1951  GAGGTCAGAG ATGAATGGCT GCAGGACCAA TTTGATCGTA GGATCGTTGT
2001  ATCAGTTCAG CCAATATACA TATCAGATGA AGATTTCGAT CCACTACAAT
2051  ACGGTATTTT ATTATACATC ACTAAGGGTT CAAAAGTGTT TAAGCCTACT
2101  AAGGAGCAAC TGGCTTTAGA CGATGCAGGT ATAACAAATG ATCAGTGGTA
2151  TTATGTTGCA TTATCTATCC CTACTGTCGT GGTGGTATTT TTCGTCTTCA
2201  TGTACTTTTT CTTATATGTC AACGGGAAAA ACAGAGATTT CACAGATGTT
2251  ACTAGAAAAG CTTTAAACAA GAAACGCCGT GTTTTGGGTA AGTTCTCGGA
2301  GATGAAGAAA TTCAAAAACA TGAAAAATCA CAAGTACACC GAATTGCCAT
2351  CTTATAAGAA AACCAGTAAA CAAAATTAG
```

FIGURE 57. *YKL077w* Protein Sequence

Nature 387:98-102 [97313270] (1997) The nucleotide sequence of Saccharomyces cerevisiae chromosome XV.

Dujon, B., Albermann, K., Aldea, M., Alexandraki, D., Ansorge, W., Arino, J., Benes, V., Bohn, C., Bolotin-Fukuhara, M., Bordonne, R., Boyer, J., Camasses, A., Casamayor, A., Casas, C., Cheret, G., et al.

YKL077W Length: 392 March 26, 1999 16:50 Type: P Check: 1732 ..

```
   1  MRFHDSILIF FSLASLYQHV HGARQVVRPK EKMTTSEEVK PWLRTVYGSQ

  51  KELVTPTVIA GVTFSEKPEE TPNPLKPWVS LEHDGRPKTI KPEINKGRTK

 101  KGRPDYSTYF KTVSSHTYSY EELKAHNMGP NEVFVEEEYI DEDDTYVSLN

 151  PIVRCTPNLY FNKGLAKDIR SEPFCTPYEN SRWKVDKTYF VTWYTRFFTD

 201  ENSGKVADKV RVHLSYVKEN PVEKGNYKRD IPATFFSSEW IDNDNGLMPV

 251  EVRDEWLQDQ FDRRIVVSVQ PIYISDEDFD PLQYGILLYI TKGSKVFKPT

 301  KEQLALDDAG ITNDQWYYVA LSIPTVVVVF FVFMYFFLYV NGKNRDFTDV

 351  TRKALNKKRR VLGKFSEMKK FKNMKNHKYT ELPSYKKTSK QN
```

FIGURE 58. ***YGR046w*** **DNA Sequence**

Sequence contains 599bp of 5' promoter sequence.
Symbols: 1 to: 1757 from: chr7.gcg ck: 9962, 584290 to: 586046
Chromosome VII Sequence
Nature 387:81-84 [97313265] (1997) The nucleotide sequence of
Saccharomyces cerevisiae chromosome VII.
Tettelin, H., Agostoni Carbone, M. L., Albermann, K., Albers, M.,
Arroyo, J., Backes, U., Barreiros, T., Bertani, I., Bjourson, A. J., . . .

gcgseq.tmp.228 Length: 1757 March 26, 1999 16:44 Type: N Check: 9449 ..

```
   1  TCTCACTCCG GCGGCCATTT TACGTGACGA AGCATCCCTT ACAACAGAAA
  51  GAAGAAAAAA GATATGCCGC TTTGCGGTTT CTTTCTGGCA ATGTATGCAC
 101  TCATAATGCT ACTCGTTTAC CCACTATCCC TGTCCAAACT AAAGAGGGAG
 151  GAAAGCACTT TTTGCATTTA CACATCGTAG ATTATAAAAT GATCGTTAAC
 201  AGGCGCTTGT GATTTTGAAT TTAAGAAATG TGGACTAGAG AAGTCTTAAA
 251  TCGCCAATGC TGTACCAGAC TCTCTATAGC ATCTAAACAC GAAATTCAAC
 301  TGTTATCTTA GTTTTTCACT TACCAGTAGC GCGCTTGTTA TTCCCACGTT
 351  ATTATTTGCC CCCACATCAT AGGTCAAGTG ACCTTCTCTT ACCCGACATG
 401  AATAAAGAAA AGAAAAGAAA TCATACCCTT CAGCCTGTTT AGCCATAAAT
 451  AGTAAAGAGT AGATGTTTCG ACGGACTAAA TAATGTGAAA AAGGTTCTAA
 501  AACCTTCAAA ACAATTAAAC TTGAGAAACG TTGCTATAGG ATTGAGCTAA
 551  TAATTTGAAT TAATAGGAGC TGCTTTTTAC TTTGATATAT CCTGAAGTTA
 601  TGTTACGAGT TTCTGAAAAT GGTCTACGGT TTCTGCTGAA ATGCCATTCA
 651  ACGAACGTAA GCATGTTTAA TAGGCTTCTG AGTACTCAAA TAAAGGAGGG
 701  GAGAAGTTCC ATAGATGATG CTGGCATTAT CCCCGATGGA ACTATTAACG
 751  AAAGGCCGAA TCACTACATC GAGGGAATTA CTAAAGGCAG TGATCTGGAC
 801  CTCTTGGAAA AAGGTATAAG AAAAACTGAC GAAATGACTT CCAATTTTAC
 851  AAATTATATG TACAAGTTTC ACAGATTGCC CCCCAACTAT GGAAGTAACC
 901  AGCTCATTAC TATCGATAAG GAACTTCAAA AGGAACTGGA TGGGGTAATG
 951  TCATCCTTCA AAGCTCCGTG CCGGTTTGTA TTTGGTTACG GCTCAGGAGT
1001  TTTCGAACAA GCGGGATATT CCAAAAGTCA TAGCAAACCT CAAATCGATA
1051  TAATCTTGGG CGTCACATAT CCATCACATT TTCACTCTAT TAATATGAGG
1101  CAGAATCCGC AACATTATTC AAGTTTGAAA TACTTCGGTT CCGAGTTCGT
1151  GTCTAAATTT CAACAGATCG GCGCAGGCGT ATATTTTAAT CCATTTGCAA
1201  ATATAAATGG ACACGACGTA AAATATGGGG TGGTTTCTAT GGAAACACTT
1251  TTAAAGGACA TAGCTACTTG GAATACATTC TATTTAGCAG GACGACTACA
1301  AAAGCCTGTA AAAATATTGA AGAATGATTT GAGAGTGCAA TATTGGAACC
1351  AATTAAACTT AAAAGCTGCA GCTACTTTGG CCAAACATTA CACCTTAGAG
1401  AAAAATAACA ATAAGTTTGA CGAATTCCAA TTTTACAAGG AGATCACTGC
1451  CTTAAGTTAT GCAGGTGATA TTAGATACAA ACTGGGTGGA GAAAATCCCG
1501  ACAAAGTTAA CAACATTGTT ACCAAAAACT TTGAAAGATT TCAAGAGTAT
1551  TACAAGCCGA TTTACAAAGA AGTGGTCCTA AATGATTCAT TTTATCTTCC
1601  AAAAGGGTTC ACATTAAAGA ATACTCAGAG ACTTTTGCTC AGCCGTATTA
1651  GTAAATCAAG TGCATTACAA ACTATTAAAG GTGTTTTCAC AGCTGGAATC
1701  ACAAAGTCAA TTAAGTATGC TTGGGCCAAA AAACTAAAAT CGATGAGGAG
1751  AAGCTAG
```

FIGURE 59. *YGR046w* Protein Sequence

Nature 387:81-84 [97313265] (1997) The nucleotide sequence of Saccharomyces cerevisiae chromosome VII.

Tettelin, H., Agostoni Carbone, M. L., Albermann, K., Albers, M., Arroyo, J., Backes, U., Barreiros, T., Bertani, I., Bjourson, A. J., Bruckner, M., Bruschi, C. V., Carignani, G., Castagnoli, L., Cerdan, et al.

YGR046W Length: 385 March 26, 1999 16:46 Type: P Check: 4137 ..

```
   1  MLRVSENGLR FLLKCHSTNV SMFNRLLSTQ IKEGRSSIDD AGIIPDGTIN

  51  ERPNHYIEGI TKGSDLDLLE KGIRKTDEMT SNFTNYMYKF HRLPPNYGSN

 101  QLITIDKELQ KELDGVMSSF KAPCRFVFGY GSGVFEQAGY SKSHSKPQID

 151  IILGVTYPSH FHSINMRQNP QHYSSLKYFG SEFVSKFQQI GAGVYFNPFA

 201  NINGHDVKYG VVSMETLLKD IATWNTFYLA GRLQKPVKIL KNDLRVQYWN

 251  QLNLKAAATL AKHYTLEKNN NKFDEFQFYK EITALSYAGD IRYKLGGENP

 301  DKVNNIVTKN FERFQEYYKP IYKEVVLNDS FYLPKGFTLK NTQRLLLSRI

 351  SKSSALQTIK GVFTAGITKS IKYAWAKKLK SMRRS
```

FIGURE 60. ***YJR041c*** **DNA Sequence**

This sequence includes 1000bp of 5' promoter sequence.
Symbols: 1 to: 4525 from: chr10.gcg /rev ck: 4711, 509927 to: 514451
Chromosome X Sequence
EMBO J. 15:2031-2049 [8641269] (1996) Complete nucleotide sequence of Saccharomyces cerevisiae chromosome X.
Galibert, F., Alexandraki, D., Baur, A., Boles, E., Chalwatzis, N., Chuat, J. C., Coster, F., Ziepluch, C., De Haan, M., Domdey, H., . . .

gcgseq.tmp.25123 Length: 4525 March 26, 1999 11:33 Type: N Check: 4481
..

```
   1  TACCTGCTGT AGAATCCTTC ACTGAAAACA CTTGTTCAAT ATATTCTTCA
  51  TCTGGTTCAC CGTCTGATCT ATTAATCCAG TTTAGCAATG ACTCAATAAA
 101  CTCTGATCTG TTCTCCTCTA CATCCTGACC ATCTAATATG AAGTACATTG
 151  TCCTCAGACA GTTTAAAACG GTTAAAGATT CTTCCAACTC ATAAAATCGG
 201  TTCACTCTTC CATCCTGATC CTTGACTCTA CCAATAAACA CTTCCAATTC
 251  ATTCAGAATC GCCTCCATGG CCAGATTTAC TGTTGCATTA TGCTCCTTCG
 301  CGAAATTAGA ATTAACAACT CCAATCGTTG GTACATTAAA CACTCTGTCA
 351  TCACCTAAAT CACGGTAAAT TTCAAATAAA CCTGATACGT ATGCAGAAAA
 401  CTCTTTGCTG GTATCTAATC TAGGAATTCT AACAGGATAA AGCTTATATT
 451  TATCTTTTGC AGTTATGAAT GCCATATTTT GGTAAGAAAG TGGCCCCAGC
 501  TTGAACTTTA AAGGCATCTT GTCGCCATTT TTTTCAATCG GTTGATCATT
 551  TACAGTCATA GGGACCAGGA TAGCCCCGCT GACTGGGTCC CTTTTATATA
 601  GTTGTTCTTC TTCATCGGTC TTGTTATTAC TAAGTTGCGC CGTTCCGTCG
 651  TCCAAAAAAT CAAATTGATC GACGTCCATA AGTAATCGAT TTGAATCATC
 701  GATTGTCATA TCTGATAATT GCGTTCTGGC TCACGCTTAT TGACTCAACT
 751  CAAGACCGTA AGTTCAATGT TTTCTATACA ACTACAATTT GTACAAGGCT
 801  TGACTTCCAT CCAACTAAAA AACCTCTCCG TCGTGCGCGA TCTGAAAAAT
 851  TTCACTTAGC TCATCTCAAA ATGATCGCTA AGAGGGCACT TGGTCACAAC
 901  TACAGAATTG TTTACTAGCA TAGGAACATC TCTGTCTAAG ATTTAGCTTG
 951  CCATCAATTA TCTTTGGAAA AACAGAGAGT ATACTGCACT TTTTGATAAT
1001  ATGGGTGATC TTACAGAAGA ACTATCTATC CCAGACAATG CCCAAGATTT
1051  GTCGAAATTA CTACGTTCGA CGAGCACAAA ACCCCATCAA ATTGCCGAGA
1101  TAGTTTCAAA ATTTGATAAA TTAGAAACCT ACTTTCCAAA AAAAGAAATT
1151  TTCGTCTTAG ATTTACTCAT TGATAGGCTC AACAATGGAA ATTTGGATGA
1201  TTTTAAGACC AGTGAACATA CTTGGATAAT TTTCACGAGA TTATTAGATG
1251  CTATTAATGA TCCAATTTCG ATAAAAAAAC TACTCAAAAA ATTGAAGACT
1301  GTGCCTGTAA TGATAAGAAC ATTTTTCCTT TGGCCTAAAG ACAAATTACT
1351  TACACGTAGC GTTTCGTTTA TAAAAGCATT TTTTGCGATT AATGACTACT
1401  TGATTGTCAA TTTTTCTGTT GAAGAGTCTT TTCAACTTTT AGAACATGCC
1451  ATAAATGGAT TAAGTTCGTG CCCGACGACT GACTTTGCGC TTTCATACTT
1501  GCAAGATGCC TGCAATCTAA CTCATGTTGA CAATATTACT ACAACGGATA
1551  ACAAAATTGC TACTTGTTAC TGCAAGCATA TGCTACTACC AAGTTTAAGA
1601  TATTTCGCAC AGACCAAAAA TTCTGCATCT TCAAACCAAT CCTTCATTCG
1651  TCTATCTCAT TTTATGGGAA AGTTCCTTTT ACAACCACGC ATAGATTACA
1701  TGAAATTAAA TAAAAAGTTT GTCCAAGAGA ATGCGTCCGA AATTACCGAC
1751  GATATGGCTT ATTATTATTT TGCCACTTTC GTCACTTTCT TATCAAAAGA
1801  CAATTTTGCT CAACTAGAAG TCATCTTTAC AATTTTAGGT GCCAAGAAAC
1851  CTAGTTTAGA ATGCAGATTT CTGAATCTTT TATCGGAATC GAAGAAAACC
1901  GTATCTCAAG AGTTCCTTGA AGCATTATTG CTTGAAATGT TAGCGTCGAC
1951  TGATGAATCT GGAGTGTTAT CATTAATACC AATTATCCTT AAATTGGATA
2001  TCGAGGTTGC TATTAAACAT ATTTTTCGGT TACTTGAATT GATTCAGCTC
2051  GAAAATTTGA ACGATCCTCT CTTTTCCTCT CATATTTGGG ATTTAATAAT
2101  CCAATCACAC GCTAACGCAA GGGAATTATC AGATTTTTTT GCCAAAATAA
2151  ATGAGTACTG TTCCAGAAAA GGACCCGATT CCTATTTTTT GATAAATCAT
2201  CCTGCATATG TCAAGTCTAT AACGAAGCAA TTGTTCACTT TATCTTCTTT
2251  ACAATGGAAA AATCTATTGC AAGCTTTACT TGACCAAGTC AATCACGATT
2301  CCACCAACAG GGTTCCTTTA TATTTAATAC GCATATGCTT GGAGGGACTA
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2351  TCAGAGGGCG CATCGCGCGC AACTCTCGAT GAGGTAAAGC CTATTTTATC
2401  TCAAGTATTT ACTTTGGAAT CATTTAATAA CAGTCTTCAA TGGGACCTAA
2451  AGTATCATAT AATGGAAGTC TACGATGATA TTGTCCCTGC AGAGGAACTA
2501  GAAAAAATCG ATTACGTGTT ATCTTCTAAT ATTTTTGATA CTACATCGGC
2551  TGATGTTGAA GAACTGTTCT TTTATTGCTT CAAATTGAGA GAATATATTT
2601  CGTTCGATCT TTCTGATGCA AAAAAAAAAT TCATGAGGCA CTTTGAAATC
2651  CTTGACGAAG AAAGAAAGTC AAACTTATCA TACTCTGTTG TGTCCAAATT
2701  TGCAACATTA GTAAACAACA ACTTTACAAG AGAACAAATT TCTTCTTTAA
2751  TTGATTCATT ACTATTGAAC TCGACAAATT TATCTTCGTT ATTAAAAAAT
2801  GATGACATTT TTGAGGAGAC AAATATCACG TACGCTTTAA TAAACAAGCT
2851  TGCTTCATCA TACCATCAAA CCTTCGCTCT AGAAGCTTTG ATTCAAATTC
2901  CTATCCAATG CATCAACAAA AACGTTAGAG TGGCTCTCAT TAACAATCTA
2951  ACATGCGAAT CATTTTGCCT TGATTCCGCT ACTAGAGAAT GCCTCCTTCA
3001  TTTATTGTCA AGCCCGACCT TCAAGAGCAA CATTGAAACA AATTTCTACG
3051  AATTATGTGA GAAAACAATA ATGAGCCCCG AAATGGCCAT TTCAGAGACA
3101  GGTGATGAAA AAAAGGAAAT AGAAGACAAA ATATCTATTT TCGAAAAAGT
3151  TTGGACTAAT CATCTGTCAC AGGCAAAGGA GCCTGTGAGT GAGAAGTTCT
3201  TAGAATCTGG TTACGATATC GTTAAACAGT CAATGTCATT GTCCAATGGT
3251  GATAGCAAAC TAATTATCGC CGGGTTTACT ATCGCAAAAT TTTTGAAACC
3301  AGATAACAAG CATAGAGATA TACAAGGTAT GGCAATTAGC TATGCTGTTA
3351  AAATTTTGGA AAACTACTCT GAAAATTTTG AATCTGAAAC AATTCCCCTT
3401  TTCAGAATAT CAATGTCTAC ATTGTACAAG ATTATAACGA CCGGACAAGG
3451  CGATATTTCT AAGCATAAAT CGAGAATTCT GGATATATTT TCCAAAATTA
3501  TGCTTCGATA TCATTCTAAA AAAGTGTACC ATGCGCCAGA AGAACAGGAA
3551  ATGTTTTTGG TTCATTCACT CCTTACAGAA AACAAGTTGG AGTATATTTT
3601  TGCAGAGTAC TTAAATATTG AGCATACAGA TAAGTGCGAT TCTGCCTTGG
3651  GGTTCTGCTT GGAAGAAAGT CTTAAACAAG GTCCTGATGC GTTTAACCGC
3701  CTGCTCTGGA ACAGTGCTAA ATCGTTTTCC ACCATTAGCC AACCTTGTGC
3751  TGAAAAATTT GTGAGAGTTT TTATCATAAT GTCAAAAAGG ATTGCAAGAG
3801  ACAATAACCT TGGTCATCAC CTATTTGTGA TAGCTTTACT TGAAGCCTAC
3851  ACCTATTGTG ATATAGAAAA ATTTGGCTAC AAGTCATACT TGCTACTGTT
3901  CAATGCTATC AAGGAGTTCT TAGTATCGAA ACCATGGCTA TTCAGCCAAT
3951  ACTGTATTGA AATGCTGCTT CCTTTCTGTT TAAAAACTCT CGCTTTTATA
4001  GTAAACCATG AGTCAACGGA TGAAATCAAT GAAGGCTTTA TTAACATCAT
4051  CGAAGTGATA GATCATATGC TATTAGTTCA CAGGTTTAAA TTTTCCAATC
4101  GTCACCATTT GTTTAACTCC GTTCTTTGCC AGATACTAGA AATAATAGCA
4151  ATTCATGATG GTACATTGTG TGCAAATTCA GCAGACGCCG TAGCCAGACT
4201  AATAACGAAC TACTGCGAGC CTTATAATGT ATCAAACGCT CAAAATGGGC
4251  AGAAAAATAA CTTAAGCTCA AAGATAAGTT TGATAAAGCA GTCCATCAGA
4301  AAAAATGTAC TTGTGGTTCT AACGAAATAT ATACAGTTGT CTATTACGAC
4351  GCAGTTCAGT TTAAACATAA AAAAGAGTCT GCAGCCCGGT ATTCATGCGA
4401  TTTTTGATAT ATTATCTCAG AACGAGTTGA ATCAATTGAA CGCTTTCCTT
4451  GACACACCTG GGAAACAATA TTTCAAAGCA CTTTACCTCC AATACAAAAA
4501  GGTTGGTAAA TGGCGCGAAG ATTAA
```

FIGURE 60 (cont).

FIGURE 61. *YJR041c* Protein Sequence

EMBO J. 15:2031-2049 [8641269] (1996) Complete nucleotide sequence of Saccharomyces cerevisiae chromosome X.
Galibert, F., Alexandraki, D., Baur, A., Boles, E., Chalwatzis, N., Chuat, J. C., Coster, F., Cziepluch, C., De Haan, M., Domdey, H., Durand, P., Entian, K. D., Gatius, M., Goffeau, A., Grivell, L. A., et al.

YJR041C Length: 1174 March 26, 1999 11:35 Type: P Check: 5083 ..

```
   1  MGDLTEELSI PDNAQDLSKL LRSTSTKPHQ IAEIVSKFDK LETYFPKKEI
  51  FVLDLLIDRL NNGNLDDFKT SEHTWIIFTR LLDAINDPIS IKKLLKKLKT
 101  VPVMIRTFFL WPKDKLLTRS VSFIKAFFAI NDYLIVNFSV EESFQLLEHA
 151  INGLSSCPTT DFALSYLQDA CNLTHVDNIT TTDNKIATCY CKHMLLPSLR
 201  YFAQTKNSAS SNQSFIRLSH FMGKFLLQPR IDYMKLNKKF VQENASEITD
 251  DMAYYYFATF VTFLSKDNFA QLEVIFTILG AKKPSLECRF LNLLSESKKT
 301  VSQEFLEALL LEMLASTDES GVLSLIPIIL KLDIEVAIKH IFRLLELIQL
 351  ENLNDPLFSS HIWDLIIQSH ANARELSDFF AKINEYCSRK GPDSYFLINH
 401  PAYVKSITKQ LFTLSSLQWK NLLQALLDQV NHDSTNRVPL YLIRICLEGL
 451  SEGASRATLD EVKPILSQVF TLESFNNSLQ WDLKYHIMEV YDDIVPAEEL
 501  EKIDYVLSSN IFDTTSADVE ELFFYCFKLR EYISFDLSDA KKKFMRHFEI
 551  LDEERKSNLS YSVVSKFATL VNNNFTREQI SSLIDSLLLN STNLSSLLKN
 601  DDIFEETNIT YALINKLASS YHQTFALEAL IQIPIQCINK NVRVALINNL
 651  TCESFCLDSA TRECLLHLLS SPTFKSNIET NFYELCEKTI MSPEMAISET
 701  GDEKKEIEDK ISIFEKVWTN HLSQAKEPVS EKFLESGYDI VKQSMSLSNG
 751  DSKLIIAGFT IAKFLKPDNK HRDIQGMAIS YAVKILENYS ENFESETIPL
 801  FRISMSTLYK IITTGQGDIS KHKSRILDIF SKIMLRYHSK KVYHAPEEQE
 851  MFLVHSLLTE NKLEYIFAEY LNIEHTDKCD SALGFCLEES LKQGPDAFNR
 901  LLWNSAKSFS TISQPCAEKF VRVFIIMSKR IARDNNLGHH LFVIALLEAY
 951  TYCDIEKFGY KSYLLLFNAI KEFLVSKPWL FSQYCIEMLL PFCLKTLAFI
1001  VNHESTDEIN EGFINIIEVI DHMLLVHRFK FSNRHHLFNS VLCQILEIIA
1051  IHDGTLCANS ADAVARLITN YCEPYNVSNA QNGQKNNLSS KISLIKQSIR
1101  KNVLVVLTKY IQLSITTQFS LNIKKSLQPG IHAIFDILSQ NELNQLNAFL
1151  DTPGKQYFKA LYLQYKKVGK WRED
```

FIGURE 62. *HES1* DNA Sequence

DNA sequence includes 1089bp 5' promoter sequence.
Symbols: 1 to: 2394 from: chr15.gcg ck: 9129, 780903 to: 783296
Chromosome XV Sequence
Nature 387:98-102 [97313270] (1997) The nucleotide sequence of
Saccharomyces cerevisiae chromosome XV.
Dujon, B., Albermann, K., Aldea, M., Alexandraki, D., Ansorge, W.,
Arino, J., Benes, V., Bohn, C., Bolotin-Fukuhara, M., Bordonne, R., . . .

gcgseq.tmp.10515 Length: 2394 March 26, 1999 14:35 Type: N Check: 4842
..

```
   1  CATGGCTGGA GGAAAGATTC CTATTGTAGG AATTGTGGCA TGTTTACAGC
  51  CGGAGATGGG GATAGGATTT CGTGGAGGTC TACCATGGAG GTTGCCCAGT
 101  GAAATGAAGT ATTTCAGACA GGTCACTTCA TTGACGAAAG ATCCAAACAA
 151  AAAAAATGCT TTGATAATGG GAAGGAAGAC ATGGGAATCC ATACCGCCCA
 201  AGTTTCGCCC ACTGCCCAAT AGAATGAATG TCATTATATC GAGAAGCTTC
 251  AAGGACGATT TTGTCCACGA TAAAGAGAGA TCAATAGTCC AAAGTAATTC
 301  ATTGGCAAAC GCAATAATGA ACCTAGAAAG CAATTTTAAG GAGCATCTGG
 351  AAAGAATCTA CGTGATTGGG GGTGGCGAAG TTTATAGTCA AATCTTCTCC
 401  ATTACAGATC ATTGGCTCAT CACGAAAATA AATCCATTAG ATAAAAACGC
 451  AACTCCTGCA ATGGACACTT TCCTTGATGC GAAGAAATTG GAAGAAGTAT
 501  TTAGCGAGCA AGATCCGGCC CAGCTGAAAG AATTTCTTCC CCCTAAAGTA
 551  GAGTTGCCCG AAACAGACTG TGATCAACGC TACTCGCTGG AAGAAAAAGG
 601  TTATTGCTTC GAATTCACTC TATACAATCG TAAATGAAAC CTCTCCGCCC
 651  GTATATTTTT TTTAATATGT TAAATAGTGA TAGAACTGAT AAGCCTCATT
 701  TTCTTTTATT GGGCTCCAAG ACGCGAACTG TTCGTAGGGT AACCGTTTGA
 751  CACCTAAACG ACCTTTCAGC CTCACCTGCA GTATTTCTTC AACAACGCCT
 801  GTCGCTATGT TAAATAATAG CAATCGTTTG TGATCACCAT TGTCGAATTT
 851  GACGCGCTTA AACAAAAACC ATTGTTTTGG CCTCGTTCCC TGCATTCAAC
 901  AAAAGAGCAA GGTATGCCGT CAAACAGTCG TTAAAAGAGA AGGTTTATAA
 951  ACTATCTTGT TTTGTACTTT GCTGTCCCGG ATCCAGTTGG GTCTTCTTTT
1001  CAACCTGTCT GAGTCCGATC TTTCTTTCCC TACTTGAAGC TCCATATATC
1051  TAAGTCATCT AAGTGTATCC TGCTAGATTA CAAACGAAAA TGTCTCAACA
1101  CGCAAGCTCA TCTTCTTGGA CTTCTTTTTT GAAATCGATA AGTTCGTTCA
1151  ACGGAGATCT ATCGTCTTTG TCTGCACCAC CGTTTATTCT TTCTCCCACT
1201  TCCTTAACAG AGTTTTCTCA GTATTGGGCT GAACATCCAG CTTTATTTCT
1251  GGAGCCTTCG TTGATTGATG GTGAAAACTA CAAAGATCAC TGTCCCTTTG
1301  ACCCAAATGT GGAATCAAAG GAAGTGGCGC AGATGTTGGC GGTTGTTAGG
1351  TGGTTTATTT CTACTTTGAG ATCTCAATAC TGCTCTAGAA GCGAATCGAT
1401  GGGTTCTGAA AAGAAGCCTT TGAACCCATT CTTGGGTGAG GTATTTGTTG
1451  GAAAGTGGAA AAATGATGAG CATCCAGAGT TTGGTGAAAC GGTTCTTTTA
1501  AGTGAGCAAG TTTCACATCA TCCACCTATG ACAGCATTTT CGATTTTTAA
1551  TGAAAAAAAT GATGTTTCTG TTCAAGGATA CAATCAAATT AAAACTGGTT
1601  TTACCAAAAC ATTGACGCTA ACGGTCAAAC CATACGGGCA TGTCATTTTG
1651  AAGATTAAAG ATGAGACCTA CCTGATTACA ACCCCGCCTT TGCATATCGA
1701  AGGTATTTTA GTCGCTTCTC CATTTGTTGA ATTAGGAGGC AGGTCATTCA
1751  TACAGTCATC AAATGGTATG TTATGTGTTA TAGAATTTTC AGGAAGGGGG
1801  TATTTCACAG GGAAGAAGAA CTCCTTTAAG GCAAGAATTT ACAGAAGCCC
1851  ACAAGAGCAT AGTCATAAAG AAAATGCGCT ATACCTAATC TCTGGCCAAT
1901  GGTCAGGTGT TTCAACAATT ATAAAAAAAG ACTCGCAAGT TTCACATCAG
1951  TTTTACGATT CATCGGAAAC TCCTACTGAA CATTTATTAG TTAAGCCAAT
2001  CGAAGAACAA CATCCTCTGG AAAGTAGGAG GGCATGGAAG GATGTGGCAG
2051  AAGCAATCAG ACAAGGAAAT ATTAGTATGA TAAAAAAGAC TAAGGAAGAA
2101  CTAGAAAATA AGCAAAGAGC CTTGAGAGAA CAAGAACGCG TAAAAGGTGT
2151  GGAATGGCAA AGAAGATGGT TCAAACAAGT GGACTACATG AATGAAAATA
2201  CATCAAATGA TGTAGAGAAA GCAAGTGAAG ATGATGCCTT TAGGAAATTG
2251  GCGTCCAAAC TGCAGCTTTC TGTGAAAAAT GTGCCAAGTG GGACATTGAT
2301  TGGCGGCAAA GATGATAAGA AAGATGTTTC AACCGCATTG CATTGGAGGT
```

FIGURE 63. *HES1* Protein Sequence

Nature 387:98-102 [97313270] (1997) The nucleotide sequence of Saccharomyces cerevisiae chromosome XV.

Dujon, B., Albermann, K., Aldea, M., Alexandraki, D., Ansorge, W., Arino, J., Benes, V., Bohn, C., Bolotin-Fukuhara, M., Bordonne, R., Boyer, J., Camasses, A., Casamayor, A., Casas, C., Cheret, G., et al.

YOR237W Length: 434 March 26, 1999 14:37 Type: P Check: 7501 ..

```
   1  MSQHASSSSW TSFLKSISSF NGDLSSLSAP PFILSPTSLT EFSQYWAEHP

  51  ALFLEPSLID GENYKDHCPF DPNVESKEVA QMLAVVRWFI STLRSQYCSR

 101  SESMGSEKKP LNPFLGEVFV GKWKNDEHPE FGETVLLSEQ VSHHPPMTAF

 151  SIFNEKNDVS VQGYNQIKTG FTKTLTLTVK PYGHVILKIK DETYLITTPP

 201  LHIEGILVAS PFVELGGRSF IQSSNGMLCV IEFSGRGYFT GKKNSFKARI

 251  YRSPQEHSHK ENALYLISGQ WSGVSTIIKK DSQVSHQFYD SSETPTEHLL

 301  VKPIEEQHPL ESRRAWKDVA EAIRQGNISM IKKTKEELEN KQRALREQER

 351  VKGVEWQRRW FKQVDYMNEN TSNDVEKASE DDAFRKLASK LQLSVKNVPS

 401  GTLIGGKDDK KDVSTALHWR FDKNLWMREN EITI
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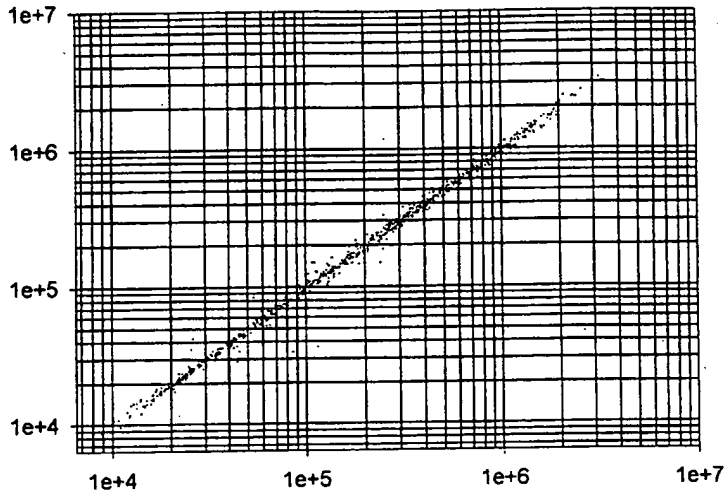

Figure 64

FIGURE 65. Rat Gene with Similarity to *YLR100w*

```
LOCUS       1397235       334 aa                                  04-FEB-1999
DEFINITION  ovarian-specific protein.
ACCESSION   1397235
PID         g1397235
DBSOURCE    locus RNU44803 accession U448031
KEYWORDS    .
SOURCE      Norway rat.
  ORGANISM  Rattus norvegicus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Rodentia; Sciurognathi; Myomorpha; Muridae;
            Murinae; Rattus.
REFERENCE   1  (residues 1 to 334)
  AUTHORS   Duan,W.R., Linzer,D.I.H. and Gibori,G.
  TITLE     Cloning and characterization of an ovarian-specific protein that
            associates with the short form of the prolactin receptor
  JOURNAL   J. Biol. Chem. 271 (26), 15602-15607 (1996)
  MEDLINE   96279080
REFERENCE   2  (residues 1 to 334)
  AUTHORS   Gibori,G. and Duan,W.R.
  TITLE     Direct Submission
  JOURNAL   Submitted (05-JAN-1996) Geula Gibori, Department of Physiology,
            University of Illinois at Chicago, Chicago, IL 60612, USA
COMMENT     Method: conceptual translation.
FEATURES             Location/Qualifiers
     source          1..334
                     /organism="Rattus norvegicus"
                     /strain="Sprague-Dawley"
                     /db_xref="taxon:10116"
                     /sex="female"
                     /tissue_type="corpus luteum"
                     /dev_stage="pregnant"
                     /cell_type="luteal"
     Protein         1..334
                     /product="ovarian-specific protein"
     CDS             1..334
                     /note="The protein can associate with the short form of
                     prolactin receptor in the rat corpus luteum."
                     /coded_by="U44803:15..1019"
ORIGIN
        1 mrkvvlitga ssgiglalcg rllaedddlh lclacrnlsk agavrdalla shpsaevsiv
       61 qmdvsnlqsv vrgaeevkrr fqrldylyln agimpnpqln lkaffcgifs rnvihmfsta
      121 eglltqndki tadgfqevfe tnlfghfili relepllchs dnpsqliwts srnakksnfs
      181 lediqhakgq epyssskyat dllnvalnrn fnqkglyssv tcpgvvmtnl tygilppfvw
      241 tlllpviwll rffahaftvt pyngaealvw lfhqkpesln pltkylsgtt glgtnyvkgq
      301 kmdvdedtae kfyktllele kqvritiqks dhhs
//
```

FIGURE 66. *DAK1* DNA Sequence

This sequence contains 1200bp of 5' promoter sequence.
Symbols: 1 to: 2955 from: chr13.gcg ck: 8335,
132275 to: 135229

Chromosome XIII Sequence
Nature 387:90-93 [97313268] (1997) The nucleotide sequence of Saccharomyces cerevisiae chromosome XIII.
Bowman, S., Churcher, C., Badcock, K., Brown, D., Chillingworth, T., Connor, R., Dedman, K., Devlin, K., Gentles, S., Hamlin, N., Hunt, S., .

gcgseq.tmp.16080 Length: 2955 March 31, 1999 09:57 Type: N Check: 5254 ..

```
   1  TAATATAAAT ACTAGTCGTT AGATGATAGT TGCTTCTTAT TCCGAAAATG
  51  AGTATGGAAG TGTTGCATAT GATAGGGCGG CTACAGTGAT GGTAAACATA
 101  AGATACTTTA GCGGGAAATT AGCAACTGGA AGTTAAATTA TCTAGACATA
 151  AGTGTGGCGG TCACGCTGAA CGCAGGAGAT CGGATAGATT GATAAGCTGA
 201  TCAAGAACAT TGATCGGTTT GTTGTTTAAA GAATGGTTTT TGAAAACGTT
 251  TGACCAGTTG CTTCTCCCAG ACGCTTACCG ATATGATGAT AAAGATAATA
 301  TCTTCAATTG AATACCCCGT GGATCAGCAC GAATAACAGA AAAAAGGGT
 351  GAAATTCACC GTAAGCATGA TACGCACTAC GTTCTTCTTA CCTTTGCCAA
 401  CGTGTTGTCT TTGACGTACG TAATTATGGG AGATCGTTGA TGATTAGCCC
 451  CAGCTCACTT TCTTCTTAAT GACTGACCCG CTACTATCAA AATTAAGGTG
 501  TCAAATATCA TGATGAATGA GGTCTCTAGG CGACTCAATT ATACATCTTT
 551  TAGAGATTTT TTTACTACTT GCAGATAATT TCTCAAGGGA TTAGATTCAA
 601  ATCTGGCTTG TCAATTACGC CCTTTTCAAG CTCATCAAAT TGCGTATGTC
 651  ATTCATGCTT CCATTAGGAA CCATAGAAGC ATGGCTGAAA TGGCAATATA
 701  CGGCTTCCCA ATTTCAACTC TAAAGTAATG GCGGTCGAAT TTAATCTATA
 751  TTTTACAGTT TTATACGTAC TTTAAAAGCA ATCAGTAAAC ACCTCTGGTG
 801  CTATTCAAGG GTTTTTTGCC TTTATTTGTT ACTGTCAATT GTCTGGCGCT
 851  GTGATAAAAA ACAAGGCATA AAGCTCCCCC GTCATGAACA TTAAGACTCG
 901  CTAGACGAGA GAGTGAAATA TAATGCATTT CCTGATTTAA ATGCGCTACA
 951  AACATGGTGT AAATCTGGCC CGGAGTGAGT GCTTGCCAAT TTGGCTTCTA
1001  AGGGAGAAAG ATCAAACCAC TCCCAATTGC GTCATTTTGA AAGAGTGGCC
1051  ACCTCGCGAG CGTCTGTCGA ACTAACTGAT GAATAAATAT ATAAGGAGAA
1101  AATCACTTCA ACTTCGCTAC AAGTAGTCAC TATTTGTAGC AACTGTAAAC
1151  GAACACATCA AAGAATAAGA TTACATTCTA TATCTAAGAC TAAATTTTAA
1201  ATGTCCGCTA AATCGTTTGA AGTCACAGAT CCAGTCAATT CAAGTCTCAA
1251  AGGGTTTGCC CTTGCTAACC CCTCCATTAC GCTGGTCCCT GAAGAAAAAA
1301  TTCTCTTCAG AAAGACCGAT TCCGACAAGA TCGCATTAAT TTCTGGTGGT
1351  GGTAGTGGAC ATGAACCTAC ACACGCCGGT TTCATTGGTA AGGGTATGTT
1401  GAGTGGCGCC GTGGTTGGCG AAATTTTTGC ATCCCCTTCA ACAAAACAGA
1451  TTTTAAATGC AATCCGTTTA GTCAATGAAA ATGCGTCTGG CGTTTTATTG
1501  ATTGTGAAGA ACTACACAGG TGATGTTTTG CATTTTGGTC TGTCCGCTGA
1551  GAGAGCAAGA GCCTTGGGTA TTAACTGCCG CGTTGCTGTC ATAGGTGATG
1601  ATGTTGCAGT TGGCAGAGAA AAGGGTGGTA TGGTTGGTAG AAGAGCATTG
1651  GCAGGTACCG TTTTGGTTCA TAAGATTGTA GGTGCCTTCG CAGAAGAATA
1701  TTCTAGTAAG TATGGCTTAG ACGGTACAGC TAAAGTGGCT AAAATTATCA
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```
1751  ACGACAATTT GGTGACCATT GGATCTTCTT TAGACCATTG TAAAGTTCCT
1801  GGCAGGAAAT TCGAAAGTGA ATTAAACGAA AAACAAATGG AATTGGGTAT
1851  GGGTATTCAT AACGAACCTG GTGTGAAAGT TTTAGACCCT ATTCCTTCTA
1901  CCGAAGACTT GATCTCCAAG TATATGCTAC CAAAACTATT GGATCCAAAC
1951  GATAAGGATA GAGCTTTTGT AAAGTTTGAT GAAGATGATG AAGTTGTCTT
2001  GTTAGTTAAC AATCTCGGCG GTGTTTCTAA TTTTGTTATT AGTTCTATCA
2051  CTTCCAAAAC TACGGATTTC TTAAAGGAAA ATTACAACAT AACCCCGGTT
2101  CAAACAATTG CTGGCACATT GATGACCTCC TTCAATGGTA ATGGGTTCAG
2151  TATCACATTA CTAAACGCCA CTAAGGCTAC AAAGGCTTTG CAATCTGATT
2201  TTGAGGAGAT CAAATCAGTA CTAGACTTGT TGAACGCATT TACGAACGCA
2251  CCGGGCTGGC CAATTGCAGA TTTTGAAAAG ACTTCTGCCC CATCTGTTAA
2301  CGATGACTTG TTACATAATG AAGTAACAGC AAAGGCCGTC GGTACCTATG
2351  ACTTTGACAA GTTTGCTGAG TGGATGAAGA GTGGTGCTGA ACAAGTTATC
2401  AAGAGCGAAC CGCACATTAC GGAACTAGAC AATCAAGTTG GTGATGGTGA
2451  TTGTGGTTAC ACTTTAGTGG CAGGAGTTAA AGGCATCACC GAAAACCTTG
2501  ACAAGCTGTC GAAGGACTCA TTATCTCAGG CGGTTGCCCA AATTTCAGAT
2551  TTCATTGAAG GCTCAATGGG AGGTACTTCT GGTGGTTTAT ATTCTATTCT
2601  TTTGTCGGGT TTTTCACACG GATTAATTCA GGTTTGTAAA TCAAAGGATG
2651  AACCCGTCAC TAAGGAAATT GTGGCTAAGT CACTCGGAAT TGCATTGGAT
2701  ACTTTATACA AATATACAAA GGCAAGGAAG GGATCATCCA CCATGATTGA
2751  TGCTTTAGAA CCATTCGTTA AAGAATTTAC TGCATCTAAG GATTTCAATA
2801  AGGCGGTAAA AGCTGCAGAG GAAGGTGCTA AATCCACTGC TACATTCGAG
2851  GCCAAATTTG GCAGAGCTTC GTATGTCGGC GATTCATCTC AAGTAGAAGA
2901  TCCTGGTGCA GTAGGCCTAT GTGAGTTTTT GAAGGGGGTT CAAAGCGCCT
2951  TGTAA
```

FIGURE 66 (cont).

FIGURE 67. *DAK1* Protein Sequence

Nature 387:90-93 [97313268] (1997) The nucleotide sequence of Saccharomyces cerevisiae chromosome XIII.

Bowman, S., Churcher, C., Badcock, K., Brown, D., Chillingworth, T., Connor, R., Dedman, K., Devlin, K., Gentles, S., Hamlin, N., Hunt, S., Jagels, K., Lye, G., Moule, S., Odell, C., Pearson, D., Rajandream, et al.

YML070W Length: 584 March 31, 1999 09:58 Type: P Check: 167 ..

```
   1  MSAKSFEVTD PVNSSLKGFA LANPSITLVP EEKILFRKTD SDKIALISGG
  51  GSGHEPTHAG FIGKGMLSGA VVGEIFASPS TKQILNAIRL VNENASGVLL
 101  IVKNYTGDVL HFGLSAERAR ALGINCRVAV IGDDVAVGRE KGGMVGRRAL
 151  AGTVLVHKIV GAFAEEYSSK YGLDGTAKVA KIINDNLVTI GSSLDHCKVP
 201  GRKFESELNE KQMELGMGIH NEPGVKVLDP IPSTEDLISK YMLPKLLDPN
 251  DKDRAFVKFD EDDEVVLLVN NLGGVSNFVI SSITSKTTDF LKENYNITPV
 301  QTIAGTLMTS FNGNGFSITL LNATKATKAL QSDFEEIKSV LDLLNAFTNA
 351  PGWPIADFEK TSAPSVNDDL LHNEVTAKAV GTYDFDKFAE WMKSGAEQVI
 401  KSEPHITELD NQVGDGDCGY TLVAGVKGIT ENLDKLSKDS LSQAVAQISD
 451  FIEGSMGGTS GGLYSILLSG FSHGLIQVCK SKDEPVTKEI VAKSLGIALD
 501  TLYKYTKARK GSSTMIDALE PFVKEFTASK DFNKAVKAAE EGAKSTATFE
 551  AKFGRASYVG DSSQVEDPGA VGLCEFLKGV QSAL
```

FIGURE 68. *PGU1* DNA Sequence

DNA sequence includes 1200bp of 5' promoter sequence.
Symbols: 1 to: 2286 from: chr10.gcg ck: 4711,
721304 to: 723589
Chromosome X Sequence
EMBO J. 15:2031-2049 [8641269] (1996) Complete nucleotide sequence of Saccharomyces cerevisiae chromosome X.
Galibert, F., Alexandraki, D., Baur, A., Boles, E., Chalwatzis, N., Chuat, J. C., Coster, F., Cziepluch, C., De Haan, M., Domdey, H., . . .

gcgseq.tmp.30022 Length: 2286 March 31, 1999 09:20 Type: N Check: 4618 ..

```
   1  ATGATTCTGA CGACCCTTTG ATAGTGGCAA TGATCAAAAA GAAAAAAAAA
  51  AGATAAGACG GTAGTGTGAA GATGACATAT AGCGCTACTC TATACTCGTC
 101  CAACTTCGAA AATAATATGT GGTCGTTGGT ACGTTCAGAT AAGAGAATAC
 151  ATCTCGCGCG TACGCATAAT TGTGGTCTAA AAAACCGCTG AAATTTTCTC
 201  AATACTGAAT AGAATCACGC TACTACGACA AGACTCGGTT ACTGTGCCTA
 251  AAATAATCCT GTGATAAACG AGTTATGTTA AACGCAGTAC AGGGGTTAAA
 301  GGGCATTGAG TTTTTGTGAG TGGAAATGCC CCCGTTATAG CTTCCAGTTT
 351  AATTACAAAT TATCAATTTA AGCAAATATA ACTGGAGGAT TGGGGAGGCG
 401  ACTAAAAATG GCTACCACGC TATTAGACAT ACAACATTGA GTATTTTATG
 451  TAATTTTGTT ACTGCTAGCA CGGCCATGCA ATTGGCAACT GAAAGCTATC
 501  TGACAACTTA AATGATTCTT AAAACAATGA CGACTATAAT CTTCTCTAAG
 551  AAGTTTCATA TCCATCTTCC TCATTATTCA GTTTCTTTTT CCTCTTGAAA
 601  GTATCGTAAA GAACAACGTC TTCACATTAG CTATTAGAAG ACCATTGAAC
 651  TACCGGATAT GAGTAAGAGT GATCTTGCCG GAGAGATAAT AGCTGCACAA
 701  AGGCCAAGGA TTAGATTAAT GGGTGCATTG TACGAAAAAA AATAGTTTAC
 751  AGTCATTTAT TCGCAATAAA TCAATTTTTT TTTCAAAAAA TATGTAAGTC
 801  TGATAAAAAA TTCTTCACTG AAGAGAGATG CTTACATTCT AATTCTTGAA
 851  TAAAAGACTC TCTAACGCTG TGAATTCTCT TTAGCTGTAA CGGAAACAGA
 901  GAGTTATTCC GTAGTCACTG AATTTTTTTT TTTTGACGCT ATTATTTAAA
 951  ACCTAGGATA TCCGTCCCAT ACAAAACGGC CACGAGTTTC AATCCCAGAA
1001  TGTACGAGTT ATAATTCTCC TAGATGCATG ATACTCGTGC ATTCGTTTAA
1051  CAATCATACC AATTTCCCAT TTTCGGGATA TTAAACATGA ACATACTTTT
1101  TTACTGTGAG AATGTGGTTT CACAATTATT CCATACAGGT ATAAAAACGC
1151  ACAGAACTTC AAACGGGAAG ACTATCTACC CACATTGATG GACAAACGCA
1201  ATGATTTCTG CTAATTCATT ACTTATTTCC ACTTTGTGCG CTTTTGCGAT
1251  CGCAACACCT TTGTCAAAAA GAGATTCCTG TACCCTAACA GGATCTTCTT
1301  TGTCTTCACT CTCAACCGTG AAAAAATGTA GCAGCATCGT TATTAAAGAC
1351  TTAACTGTCC CAGCTGGACA GACTTTAGAT TTAACTGGGT TAAGCAGTGG
1401  TACTACTGTT ACGTTTGAAG GCACAACCAC ATTTCAGTAC AAGGAATGGA
1451  GCGGCCCTTT AATTTCAATC TCAGGGTCTA AAATCAGCGT TGTTGGTGCT
1501  TCGGGACATA CCATTGATGG TCAAGGAGCA AAATGGTGGG ATGGCTTAGG
1551  TGATAGCGGT AAAGTCAAAC CGAAGTTTGT AAAGTTGGCG TTGACGGGAA
1601  CATCTAAGGT CACCGGATTG AATATTAAAA ATGCTCCACA CCAAGTCTTC
1651  AGCATCAATA AATGTTCAGA TTTAACCATC AGCGACATAA CAATTGATAT
1701  CAGAGACGGT GATTCGGCTG GTGGTCATAA TACGGATGGG TTTGATGTTG
1751  GTAGTTCTAG TAACGTCTTA ATTCAAGGAT GTACTGTTTA TAATCAGGAT
1801  GACTGTATTG CTGTGAATTC CGGTTCAACT ATTAAATTTA TGAACAACTA
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1851  CTGCTACAAT GGCCATGGTA TTTCTGTAGG TTCTGTTGGT GGCCGTTCTG
1901  ATAATACAGT CAATGGTTTC TGGGCTGAAA ATAACCATGT TATCAACTCT
1951  GACAACGGGT TGAGAATAAA AACCGTAGAA GGTGCGACAG GCACAGTCAC
2001  TAATGTCAAC TTTATCAGTA ATAAAATTAG CGGCATAAAA AGTTATGGTA
2051  TTGTTATCGA AGGCGATTAT TTGAATAGTA AGACTACTGG AACTGCTACA
2101  GGTGGCGTTC CCATTTCGAA TTTAGTAATG AAGGATATCA CCGGGAGCGT
2151  GAACTCCACA GCGAAGAGGG TTAAAATTTT GGTGAAAAAC GCTACTAACT
2201  GGCAATGGTC TGGGGTGTCA ATTACCGGTG GTTCTTCCTA TTCTGGATGT
2251  TCTGGAATCC CATCTGGATC TGGTGCAAGC TGTTAA
```

FIGURE 68 (cont).

FIGURE 69. *PGU1* Protein Sequence

EMBO J. 15:2031-2049 [8641269] (1996) Complete nucleotide sequence of Saccharomyces cerevisiae chromosome X.

Galibert, F., Alexandraki, D., Baur, A., Boles, E., Chalwatzis, N., Chuat, J. C., Coster, F., Cziepluch, C., De Haan, M., Domdey, H., Durand, P., Entian, K. D., Gatius, M., Goffeau, A., Grivell, L. A., et al.

YJR153W Length: 361 March 31, 1999 09:55 Type: P Check: 9795 ..

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   1  MISANSLLIS TLCAFAIATP LSKRDSCTLT GSSLSSLSTV KKCSSIVIKD
  51  LTVPAGQTLD LTGLSSGTTV TFEGTTTFQY KEWSGPLISI SGSKISVVGA
 101  SGHTIDGQGA KWWDGLGDSG KVKPKFVKLA LTGTSKVTGL NIKNAPHQVF
 151  SINKCSDLTI SDITIDIRDG DSAGGHNTDG FDVGSSSNVL IQGCTVYNQD
 201  DCIAVNSGST IKFMNNYCYN GHGISVGSVG GRSDNTVNGF WAENNHVINS
 251  DNGLRIKTVE GATGTVTNVN FISNKISGIK SYGIVIEGDY LNSKTTGTAT
 301  GGVPISNLVM KDITGSVNST AKRVKILVKN ATNWQWSGVS ITGGSSYSGC
 351  SGIPSGSGAS C
```

FIGURE 70. *STE18* DNA Sequence

This sequence contains 600bp of 5' promoter sequence.
Symbols: 1 to: 933 from: chr10.gcg ck: 4711,
585156 to: 586088

Chromosome X Sequence
EMBO J. 15:2031-2049 [8641269] (1996) Complete nucleotide sequence of Saccharomyces cerevisiae chromosome X.
Galibert, F., Alexandraki, D., Baur, A., Boles, E., Chalwatzis, N., Chuat, J. C., Coster, F., Cziepluch, C., De Haan, M., Domdey, H., . . .

gcgseq.tmp.6719 Length: 933 March 31, 1999 10:01 Type: N
Check: 8833 ..

```
   1  TTCGTTTCTG TCTTGTCTCC CGCTGTTACC TAATAACTTC ATGTGATCTG
  51  CTCCCCCTTC TCGTTAAATA CCACCTTTTC ATCAACCCCG TAGGGCGCGA
 101  CACGTCTAAA ATATTAACCT CTGAATACTT ATTGGGTCAA AATGAATGTT
 151  GATAACTTTC CTTTACAAAA AAAAAACTAA TAGAGTATAT GCATTTCGGT
 201  AGTGAAATAT TCGTTAATGC TAATATGCTC AGTAGTGATC CTAGATTACC
 251  AGTTTTACTG CAGCCATCGT ACAATTTTGG AACGAGTATA AAGAGAGAAA
 301  TTAAAAACGA CAAGAAATAT TCGTACTAGC TTCTCTTCCG GCTTGATGAC
 351  AGTCTTAATA TCATCTGCAA CTCTTGAAAT CTTGCTTTAT AGTCAAAATT
 401  TACGTACGCT TTTCACTATA TAATATGATT TGTCAATGTG ATGAGTGAAT
 451  GTCTCCCTGT TACCCGGTTT TCATGTTGAT TTTTGTTTCA GGCTCTAAAT
 501  GTTTGATGCA ATATTTAACA AGGAGAACAG AAATGTTTTG TGACAGCACC
 551  TGTCAATTTT AGGATAGTAG CAATCGCAAA CGTTCTCAAT AATTCTAAGA
 601  ATGACATCAG TTCAAAACTC TCCACGCTTA CAACAACCTC AGGAACAGCA
 651  ACAGCAACAG CAACAGCTTT CCTTAAAGAT AAAACAATTG AAGTTAAAAA
 701  GAATCAACGA ACTTAACAAT AAACTGAGGA AAGAACTCAG CCGTGAAAGA
 751  ATTACTGCTT CAAATGCATG TCTTACAATA ATAAACTATA CCTCGAATAC
 801  AAAAGATTAT ACATTACCAG AACTATGGGG CTACCCCGTA GCAGGATCAA
 851  ATCATTTTAT AGAGGGTTTG AAAAATGCTC AAAAAAATAG CCAAATGTCA
 901  AACTCAAATA GTGTTTGTTG TACGCTTATG TAA
```

FIGURE 71. *STE18* Protein Sequence

EMBO J. 15:2031-2049 [8641269] (1996) Complete nucleotide sequence of Saccharomyces cerevisiae chromosome X.

Galibert, F., Alexandraki, D., Baur, A., Boles, E., Chalwatzis, N., Chuat, J. C., Coster, F., Cziepluch, C., De Haan, M., Domdey, H., Durand, P., Entian, K. D., Gatius, M., Goffeau, A., Grivell, L. A., et al.

YJR086W Length: 110 March 31, 1999 10:02 Type: P Check: 6859 ..

```
  1  MTSVQNSPRL QQPQEQQQQQ QQLSLKIKQL KLKRINELNN KLRKELSRER
 51  ITASNACLTI INYTSNTKDY TLPELWGYPV AGSNHFIEGL KNAQKNSQMS
101  NSNSVCCTLM
```

FIGURE 72. *YGL198w* DNA Sequence

This sequence contains 989bp of 5' promoter sequence.
Symbols: 1 to: 1775 from: chr7.gcg ck: 9962,
122605 to: 124379

Chromosome VII Sequence
Nature 387:81-84 [97313265] (1997) The nucleotide sequence of Saccharomyces cerevisiae chromosome VII.
Tettelin, H., Agostoni Carbone, M. L., Albermann, K., Albers, M., Arroyo, J., Backes, U., Barreiros, T., Bertani, I., Bjourson, A. J., . .

gcgseq.tmp.32650 Length: 1775 March 31, 1999 10:03 Type: N Check: 2850 ..

```
   1  GAGAATTATT CGCGACTTCA GGTTATCCAA TCGTGTATGT AATCGTATGT
  51  AGGCAAAAGT AAATAGATAT GAACTACATT TTCCTGCTTT ACTTAGACTA
 101  GAGATGTGAC CTCAAAGAAT CTTCTCAAGT AGTATATCTG GAAAAGAGAG
 151  TTTGCAATAA CGACGCCCAA TTGGAAGATG GACCACCATT TAACACGATC
 201  GTTGGTCGAC TCTGCAGTAT TTCTATGCGT CCTTTCTCTA ATAACAATAT
 251  AACTTTGTTC GTCCTTGACT TCCCTGGTTA ATTTGGACAA CTTTCTGACA
 301  GCACTATCCA ATGTATTGGT GTTTGGGTCG TCCAAATCCA CATATACCAC
 351  CCCATGAATG TTGAAAGTCA CGTCTTTTGT CTCGATACCG GTGTTCTCGT
 401  TCAAGAAACA GTATTGGAAA TGTCCCTTGT ATGGAGCAGA CAATGTGATT
 451  TCACCGTGCG ACGTGTCCCT AACCGTTTTC AAAACTTCAT GTCTTTCCGG
 501  CCCGTAGATG ATAAAGTCAC CAGTCAGCTG GCTACTGGAT TGAGGGTTTC
 551  TATCACCGAA CTGGAACGAA ATGGAGAGCT CGTCACCCTT ACTCAAGTCT
 601  TCGAAGAAGC ATCTACGGCC ATAAGCTGGA AGAAGGACAT TATGGGCGGA
 651  CGCCGAGAAG AACAGGAAGC AAGCAATGAC AAACTTAGTA GCAAATGAGG
 701  CCATCCTTAT GCGTGTGTAT TTTTGTGCGG AGGGATACTA TTAAGATTGC
 751  AGTTTCACCA AGTATAGCTT TTTATTTCAT TATAAGTTTC GTGTCAAAAT
 801  GTTTAAGCGA CCCGATCTCT CAGGCTGTTT TGCACGACTT TTCTGACTTT
 851  CCTCGCGTCT TTTTTCATGA AAATTGGATT ACCCGGAGTG ATGATTTTCT
 901  CACAGTGATT TTTCGTCCCC TTTTACAATA GCAAATGAAG CTGTTTTAGC
 951  AATATTTGTA GAAAGATATG TCACAAGAGG GCAGGCAAAA TGTCATACGG
1001  AAGAGAAGAC ACTACGATTG AGCCCGACTT CATAGAACCA GATGCACCTT
1051  TGGCTGCTTC CGGGGGTGTT GCTGACAACA TAGGCGGAAC TATGCAGAAT
1101  TCAGGCAGCA GAGGGACGCT CGACGAGACT GTGCTGCAAA CACTAAAGCG
1151  AGATGTGGTG GAGATTAATT CCAGACTGAA ACAAGTGGTA TACCCGCATT
1201  TCCCCTCATT CTTTAGCCCC TCTGATGACG GGATAGGGGC GGCTGATAAC
1251  GACATTTCAG CCAATTGCGA CCTGTGGGCG CCCCTTGCGT TTATCATATT
1301  GTATTCTCTA TTTGTATCGC ATGCGCGGTC GCTGTTCTCG AGCCTATTTG
1351  TGTCTAGTTG GTTCATTTTG CTGGTGATGG CATTGCATCT GAGACTCACC
1401  AAGCCACACC AGAGGGTGTC GCTGATTTCG TACATCTCCA TTTCCGGGTA
1451  TTGCTTATTC CCACAAGTGC TGAATGCCTT AGTCTCGCAG ATACTACTTC
1501  CATTGGCCTA CCATATTGGA AAGCAAAATC GCTGGATTGT GAGGGTCCTG
1551  TCGCTCGTGA AACTGGTGGT CATGGCGCTG TGCCTGATGT GGTCTGTGGC
1601  CGCCGTTTCG TGGGTTACCA AGAGCAAGAC CATTATCGAG ATATACCTCT
1651  GGCACTCTGT CTTTTTTGGC ATGGCTGGTT GTCAACTATT TTATAACACT
1701  AGTTACATAT GTATAAAACC CAATATTCAT GGACATAGAA TTGCCTATCT
1751  CGCGAGCCAC GGCAGAAAGT TCTGA
```

FIGURE 73. *YGL198w* Protein Sequence

Nature 387:81-84 [97313265] (1997) The nucleotide sequence of Saccharomyces cerevisiae chromosome VII.

Tettelin, H., Agostoni Carbone, M. L., Albermann, K., Albers, M., Arroyo, J., Backes, U., Barreiros, T., Bertani, I., Bjourson, A. J., Bruckner, M., Bruschi, C. V., Carignani, G., Castagnoli, L., Cerdan, et al.

YGL198W Length: 261 March 31, 1999 10:05 Type: P Check: 1705 ..

```
   1  MSYGREDTTI EPDFIEPDAP LAASGGVADN IGGTMQNSGS RGTLDETVLQ
  51  TLKRDVVEIN SRLKQVVYPH FPSFFSPSDD GIGAADNDIS ANCDLWAPLA
 101  FIILYSLFVS HARSLFSSLF VSSWFILLVM ALHLRLTKPH QRVSLISYIS
 151  ISGYCLFPQV LNALVSQILL PLAYHIGKQN RWIVRVLSLV KLVVMALCLM
 201  WSVAAVSWVT KSKTIIEIYL WHSVFFGMAG CQLFYNTSYI CIKPNIHGHR
 251  IAYLASHGRK F
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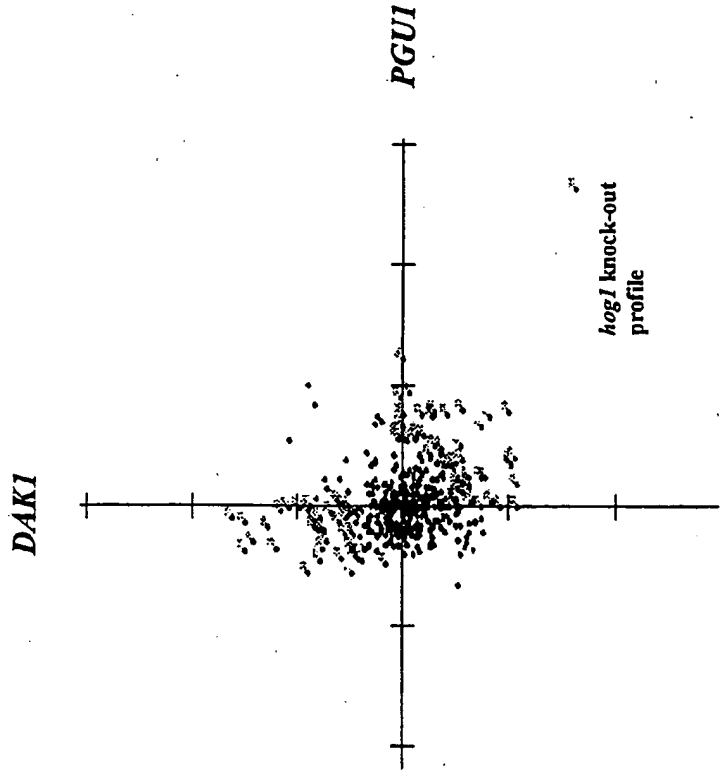

Figure 74

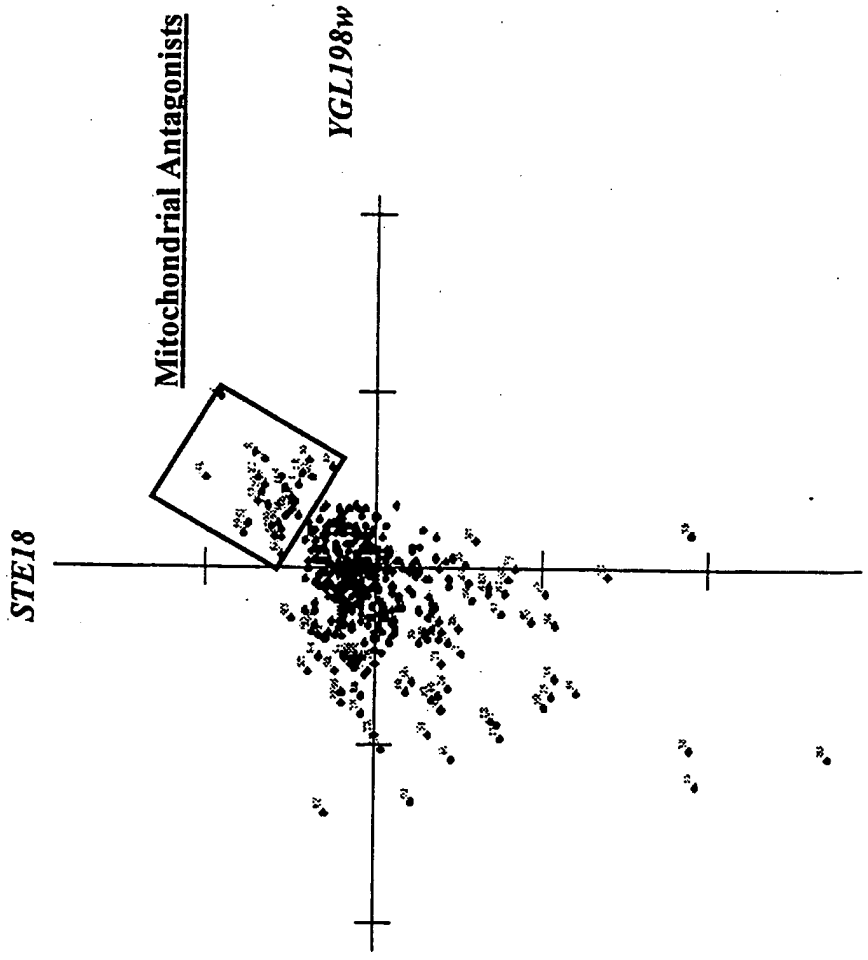

Figure 75

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(54) Title: CELL DEATH RELATED DRUG TARGETS IN YEAST AND FUNGI

(57) Abstract: The invention describes the use of nucleic acids and polypeptides which are involved in a pathway eventually leading to programmed cell death of yeast or fungi for the preparation of a medicament for treating diseases associated with yeast or fungi or for the treatment of proliferative disorders or for preventing apoptosis in certain diseases. Methods are provided to identify compounds which selectively modulate the expression or functionality of said polypeptides in the same or a parallel pathway. Also provided are compounds as well as pharmaceutical compositions, medicaments and vaccines. The invention also comprises new nucleic acid sequences, probes and primers derived thereof, expression vectors and host cells transformed with said vectors, polypeptides and antibodies raised against said polypeptides.

CELL DEATH RELATED DRUG TARGETS IN YEAST AND FUNGI

The present invention relates to the identification of genes and proteins encoded thereof from yeast and fungi whose expression is modulated upon programmed cell death and which genes, proteins or functional fragments and equivalents thereof may be used as selective targets for drugs to treat infections caused by or associated with yeast and fungi or for the treatment of proliferative disorders or for the prevention of apoptosis in certain diseases.

Invasive fungal infections (e.g. Candida spp, Aspergillus spp., Fusarium spp., Zygomycetes spp.) (Walsh, 1992) have emerged during the past two decades as important pathogens causing formidable morbidity and mortality in an increasingly diverse and progressively expanding population of immunocompromised patients. Those with the acquired immune deficiency syndrome (AIDS) constitute the most rapidly growing group of patients at risk for life-threatening mycosis. But fungal infections have also increased in frequency in several populations of other susceptible hosts, including very-low-birth-weight infants, cancer patients receiving chemotherapy, organ transplant recipients, burn patients and surgical patients with complications.

These fungal infections are not limited to humans and other mammals, but are also important in plants where they can cause diseases or cause the production of unwanted compounds (e.g. Fusarium spp., Aspergillus spp., Botritis spp., Cladosporium spp.).

Although recent advances in antifungal chemotherapy have had an impact on these mycoses, expanding populations of immunocompromised patients will require newer approaches to antifungal therapy. The discovery of novel antifungal agents is thus an essential element of any new antifungal therapy.

Classical approaches for identifying anti-fungal compounds have relied almost exclusively on inhibition of fungal or yeast growth as an endpoint. Libraries of natural products, semi-synthetic, or synthetic chemicals are screened for their ability to kill or arrest growth of the target pathogen or a related nonpathogenic model organism. These tests are cumbersome and provide no information about a compound's mechanism of action. The promising lead compounds that emerge from such screens must then be tested for possible host-toxicity and detailed mechanism of action studies must subsequently be conducted to identify the affected molecular target.

Cells from multicellular organisms can commit suicide in response to specific signals or injury by an intrinsic program of cell death. Apoptosis is a form of programmed cell death which leads to elimination of unnecessary or damaged cells. To

survive, all cells from multicellular organisms depend on the constant repression of this suicide program by signals from other cells (Raff, 1992). It has been assumed that such an altruistic form of cell survival arose with multicellularity and would have been counterselected in unicellular organisms. Recent findings indicate, however, that a similar process of cell survival also operates in single-celled eukaryotes.

It has been found that expression of the mammalian *Bax* gene triggers cell death in *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe* with morphological changes similar to apoptosis (Jürgensmeier *et al.*, 1997). However, the mechanism of *Bax* lethality in *S. cerevisiae* remains unclear.

Since it has been discovered that the mammalian *Bax* gene triggers apoptotic changes in yeast (Ligr *et al.*, 1998), this can be an indication that the molecular pathways eventually leading to programmed cell death may also be partially present in yeast cells and other unicellular eukaryotes.

It is an aim of the present invention to provide nucleic acid as well as polypeptide sequences which represent potential molecular targets for the identification of new compounds which can be used in alleviating diseases or conditions associated with yeast or fungi infections.

It is a further aim of the present invention to provide uses of these nucleic acid and amino acid molecules for the preparation of a medicament for treating diseases associated with yeast or fungi.

It is also an aim of the invention to provide pharmaceutical compositions and vaccines comprising these nucleic acids or polypeptides.

It is also an aim of the present invention to provide vectors comprising these nucleic acids, as well as host cells transfected or transformed with said vectors.

It is also an aim of the invention to provide antibodies against these polypeptides, which can be used as such, or in a composition as a medicine for treating diseases associated with yeast and fungi.

It is another aim of the invention to provide methods to selectively identify compounds capable of inhibiting or activating expression of such polypeptides in yeast or fungi infections. The nucleic acid and polypeptide molecules alternatively can be incorporated into an assay or kit to identify these compounds.

It is also an aim of the invention to provide a method of preventing infection with yeast or fungi.

It is also an aim of the invention to provide probes and primers derived from the nucleic acid sequences of the invention.

Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions

JAMES U. BOWIE,* JOHN F. REIDHAAR-OLSON, WENDELL A. LIM, ROBERT T. SAUER

An amino acid sequence encodes a message that determines the shape and function of a protein. This message is highly degenerate in that many different sequences can code for proteins with essentially the same structure and activity. Comparison of different sequences with similar messages can reveal key features of the code and improve understanding of how a protein folds and how it performs its function.

THE GENOME IS MANIFEST LARGELY IN THE SET OF PROteins that it encodes. It is the ability of these proteins to fold into unique three-dimensional structures that allows them to function and carry out the instructions of the genome. Thus, comprehending the rules that relate amino acid sequence to structure is fundamental to an understanding of biological processes. Because an amino acid sequence contains all of the information necessary to determine the structure of a protein (*1*), it should be possible to predict structure from sequence, and subsequently to infer detailed aspects of function from the structure. However, both problems are extremely complex, and it seems unlikely that either will be solved in an exact manner in the near future. It may be possible to obtain approximate solutions by using experimental data to simplify the problem. In this article, we describe how an analysis of allowed amino acid substitutions in proteins can be used to reduce the complexity of sequences and reveal important aspects of structure and function.

Methods for Studying Tolerance to Sequence Variation

There are two main approaches to studying the tolerance of an amino acid sequence to change. The first method relies on the process of evolution, in which mutations are either accepted or rejected by natural selection. This method has been extremely powerful for proteins such as the globins or cytochromes, for which sequences from many different species are known (*2–7*). The second approach uses genetic methods to introduce amino acid changes at specific positions in a cloned gene and uses selections or screens to identify functional sequences. This approach has been used to great advantage for proteins that can be expressed in bacteria or yeast, where the appropriate genetic manipulations are possible (*3, 8–11*). The end results of both methods are lists of active sequences that can be compared and analyzed to identify sequence features that are essential for folding or function. If a particular property of a side chain, such as charge or size, is important at a given position, only side chains that have the required property will be allowed. Conversely, if the chemical identity of the side chain is unimportant, then many different substitutions will be permitted.

Studies in which these methods were used have revealed that proteins are surprisingly tolerant of amino acid substitutions (*2–4, 11*). For example, in studying the effects of approximately 1500 single amino acid substitutions at 142 positions in *lac* repressor, Miller and co-workers found that about one-half of all substitutions were phenotypically silent (*11*). At some positions, many different, nonconservative substitutions were allowed. Such residue positions play little or no role in structure and function. At other positions, no substitutions or only conservative substitutions were allowed. These residues are the most important for *lac* repressor activity.

What roles do invariant and conserved side chains play in proteins? Residues that are directly involved in protein functions such as binding or catalysis will certainly be among the most conserved. For example, replacing the Asp in the catalytic triad of trypsin with Asn results in a 10^6-fold reduction in activity (*12*). A similar loss of activity occurs in λ repressor when a DNA binding residue is changed from Asn to Asp (*13*). To carry out their function, however, these catalytic residues and binding residues must be precisely oriented in three dimensions. Consequently, mutations in residues that are required for structure formation or stability can also have dramatic effects on activity (*10, 14–16*). Hence, many of the residues that are conserved in sets of related sequences play structural roles.

Substitutions at Surface and Buried Positions

In their initial comparisons of the globin sequences, Perutz and co-workers found that most buried residues require nonpolar side chains, whereas few features of surface side chains are generally conserved (*6*). Similar results have been seen for a number of protein families (*2, 4, 5, 7, 17, 18*). An example of the sequence tolerance at surface versus buried sites can be seen in Fig. 1, which shows the allowed substitutions in λ repressor at residue positions that are near the dimer interface but distant from the DNA binding surface of the protein (*9*). These substitutions were identified by a functional

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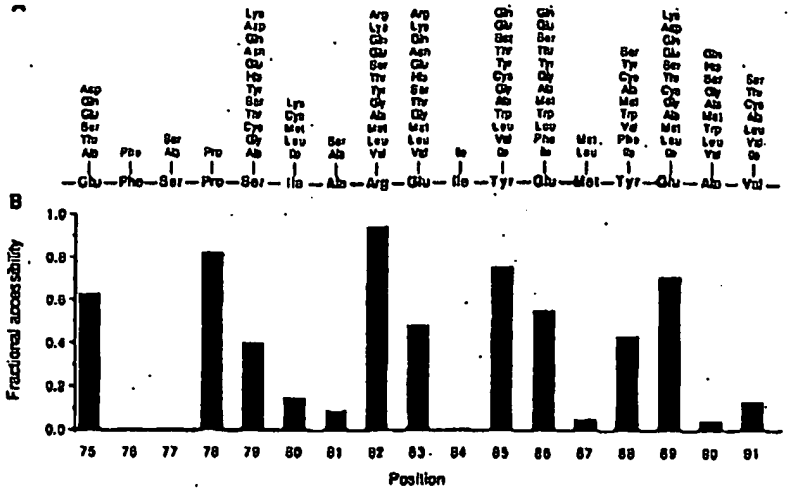
short region of λ repressor. The wild-type sequence is shown along the center line. The allowed substitutions shown above each position were identified by randomly mutating one to three codons at a time by using a cassette method and applying a functional selection (*9*). (B) The fractional solvent accessibility (*42*) of the wild-type side chain in the protein dimer (*43*) relative to the same atoms in an Ala-X-Ala model tripeptide.



selection after cassette mutagenesis. A histogram of side chain solvent accessibility in the crystal structure of the dimer is also shown in Fig. 1. At six positions, only the wild-type residue or relatively conservative substitutions are allowed. Five of these positions are buried in the protein. In contrast, most of the highly exposed positions tolerate a wide range of chemically different side chains, including hydrophilic and hydrophobic residues. Hence, it seems that most of the structural information in this region of the protein is carried by the residues that are solvent inaccessible.

Constraints on Core Sequences

Because core residue positions appear to be extremely important for protein folding or stability, we must understand the factors that dictate whether a given core sequence will be acceptable. In general, only hydrophobic or neutral residues are tolerated at buried sites in proteins, undoubtedly because of the large favorable contribution of the hydrophobic effect to protein stability (*19*). For example, Fig. 2 shows the results of genetic studies used to investigate the substitutions allowed at residue positions that form the hydrophobic core of the NH_2-terminal domain of λ repressor (*20*). The acceptable core sequences are composed almost exclusively of Ala, Cys, Thr, Val, Ile, Leu, Met, and Phe. The acceptability of many different residues at each core position presumably reflects the fact that the hydrophobic effect, unlike hydrogen bonding, does not depend on specific residue pairings. Although it is possible to imagine a hypothetical core structure that is stabilized exclusively by residues forming hydrogen bonds and salt bridges, such a core would probably be difficult to coinstruct because hydrogen bonds require pairing of donors and acceptors in an exact geometry. Thus the repertoire of possible structures that use a polar core would probably be extremely limited (*21*). Polar and charged residues are occasionally found in the cores of proteins, but only at positions where their hydrogen bonding needs can be satisfied (*22*).

The cores of most proteins are quite closely packed (*23*), but some volume changes are acceptable. In λ repressor, the overall core volume of acceptable sequences can vary by about 10%. Changes at individual sites, however, can be considerably larger. For example, as shown in Fig. 2, both Phe and Ala are allowed at the same core position in the appropriate sequence contexts. Large volume changes at individual buried sites have also been observed in phylogenetic studies, where it has been noted that the size decreases and increases at interacting residues are not necessarily related in a simple complementary fashion (*5, 7, 17*). Rather, local volume changes are accommodated by conformational changes in nearby side chains and by a variety of backbone movements.

The Informational Importance of the Core

With occasional exceptions, the core must remain hydrophobic and maintain a reasonable packing density. However, since the core is composed of side chains that can assume only a limited number of conformations (*24*), efficient packing must be maintained without steric clashes. How important are hydrophobicity, volume, and steric complementarity in determining whether a given sequence can form an acceptable core? Each factor is essential in a physical sense, as a stable core is probably unable to tolerate unsatisfied hydrogen bonding groups, large holes, or steric overlaps (*25*). However, in an informational sense, these factors are not equivalent. For example, in experiments in which three core residues of λ repressor were mutated simultaneously, volume was a relatively unimportant informational constraint because three-quarters of all possible combinations of the 20 naturally occurring amino acids had volumes within the range tolerated in the core, and yet most of these sequences were unacceptable (*20*). In contrast, of the sequences that contained only

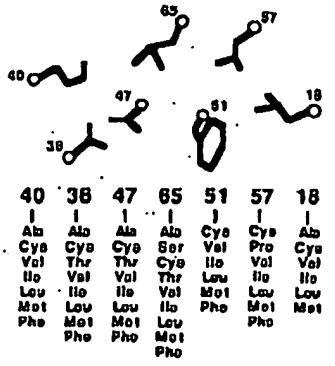


Fig. 2. Amino acid substitutions allowed in the core of λ repressor. The wild-type side chains are shown pictorially in the approximate orientation seen in the crystal structure (*43*). The lists of allowed substitutions at each position are shown below the wild-type side chains. These substitutions were identified by randomly mutating one to four residues at a time by using a cassette method and applying a functional selection (*20*). Not all substitutions are allowed in every sequence background.

the appropriate hydrophobic residues, a significant fraction were acceptable. Hence, the hydrophobicity of a sequence contains more information about its potential acceptability in the core than does the total side chain volume. Steric compatibility was intermediate between volume and hydrophobicity in informational importance.

The Informational Importance of Surface Sites

We have noted that many surface sites can tolerate a wide variety of side chains, including hydrophilic and hydrophobic residues. This result might be taken to indicate that surface positions contain little structural information. However, Bashford *et al.*, in an extensive analysis of globin sequences (*9*), found a strong bias against large hydrophobic residues at many surface positions. At one level, this may reflect constraints imposed by protein solubility, because large patches of hydrophobic surface residues would presumably lead to aggregation. At a more fundamental level, protein folding requires a partitioning between surface and buried positions. Consequently, to achieve a unique native state without significant competition from other conformations, it may be important that some sites have a decided preference for exterior rather than interior positions. As a result, many surface sites can accept hydrophobic residues individually, but the surface as a whole can probably tolerate only a moderate number of hydrophobic side chains.

Identification of Residue Roles from Sets of Sequences

Often, a protein of interest is a member of a family of related sequences. What can we infer from the pattern of allowed substitutions at positions in sets of aligned sequences generated by genetic or phylogenetic methods? Residue positions that can accept a number of different side chains, including charged and highly polar residues, are almost certain to be on the protein surface. Residue positions that remain hydrophobic, whether variable or not, are likely to be buried within the structure. In Fig. 3, those residue positions in λ repressor that can accept hydrophilic side chains are shown in orange and those that cannot accept hydrophilic side chains are shown in green. The obligate hydrophobic positions define the core of the structure, whereas positions that can accept hydrophilic side chains define the surface.

Functionally important residues should be conserved in sets of active sequences, but it is not possible to decide whether a side chain is functionally or structurally important just because it is invariant or conserved. To make this distinction requires an independent assay of protein folding. The ability of a mutant protein to maintain a stably folded structure can often be measured by biophysical techniques, by susceptibility to intracellular proteolysis (*26*), or by binding to antibodies specific for the native structure (*27, 28*). In the latter cases, it is possible to screen proteins in mutated clones for the ability to fold even if these proteins are inactive. Sets of sequences that allow formation of a stable structure can then be compared to the sets that allow both folding and function, with the active site or binding residues being those that are variable in the set of stable proteins but invariant in the set of functional proteins. The DNA-binding residues of Arc repressor were identified by this method (*8*). The receptor-binding residues of human growth hormone were also identified by comparing the stabilities and activities of a set of mutant sequences (*28*). However, in this case, the mutants were generated as hybrid sequences between growth hormone and related hormones with different binding specificities.

Implications for Structure Prediction

At present, the only reliable method for predicting a low-resolution tertiary structure of a new protein is by identifying sequence similarity to a protein whose structure is already known (*29, 30*). However, it is often difficult to align sequences as the level of sequence similarity decreases, and it is sometimes impossible to detect statistically significant sequence similarity between distantly related proteins. Because the number of known sequences is far greater than the number of known structures, it would be advantageous to increase the reach of the available structural information by improving methods for detecting distant sequence relations and for subsequently aligning these sequences based on structural principles. In a normal homology search, the sequence database is scanned with a single test sequence, and every residue must be weighted equally. However, some residues are more important than others and should be weighted accordingly. Moreover, certain regions of the protein are more likely to contain gaps than others. Both kinds of information can be obtained from sequence sets, and several techniques have

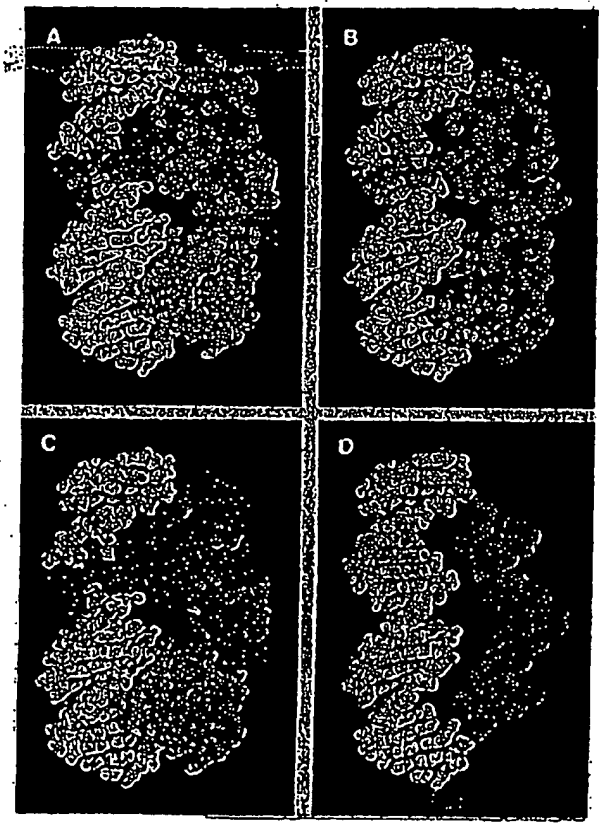


Fig. 3. Tolerance of positions in the NH_2-terminal domain of λ repressor to hydrophilic side chains. The complex (*43*) of the repressor dimer (blue) and operator DNA (white) is shown. In (A), positions that can tolerate hydrophilic side chains are shown in orange. The same side chains are shown in (B) without the remaining protein atoms. In (C), positions that require hydrophobic or neutral side chains are shown in green. These side chains are shown in (D) without the remaining protein atoms. About three-fourths of the 92 side chains in the NH_2-terminal domain are included in both (B) and (D). The remaining positions have not been tested. Data are from (*9, 14, 20, 27, 44*).

been used to combine such information into more appropriately weighted sequence searches and alignments (*31*). These methods were used to align the sequences of retroviral proteases with aspartic proteases, which in turn allowed construction of a three-dimensional model for the protease of human immunodeficiency virus type 1 (*29*). Comparison with the recently determined crystal structure of this protein revealed reasonable agreement in many areas of the predicted structure (*32*).

The structural information at most surface sites is highly degenerate. Except for functionally important residues, exterior positions seem to be important chiefly in maintaining a reasonably polar surface. The information contained in buried residues is also degenerate, the main requirement being that these residues remain hydrophobic. Thus, at its most basic level, the key structural message in an amino acid sequence may reside in its specific pattern of hydrophobic and hydrophilic residues. This is meant in an informational sense. Clearly, the precise structure and stability of a protein depends on a large number of detailed interactions. It is possible, however, that structural prediction at a more primitive level can be accomplished by concentrating on the most basic informational aspects of an amino acid sequence. For example, amphipathic patterns can be extracted from aligned sets of sequences and used, in some cases, to identify secondary structures.

If a region of secondary structure is packed against the hydrophobic core, a pattern of hydrophobic residues reflecting the periodicity of the secondary structure is expected (*33, 34*). These patterns can be obscured in individual sequences by hydrophobic residues on the protein surface. It is rare, however, for a surface position to remain hydrophobic over the course of evolution. Consequently, the amphipathic patterns expected for simple secondary structures can be much clearer in a set of related sequences (*6*). This principle is illustrated in Fig. 4, which shows helical hydrophobic moment plots for the Antennapedia homeodomain sequence (Fig. 4A) and for a composite sequence derived from a set of homologous homeodomain proteins (Fig. 4B) (*35*). The hydrophobic moment is a simple measure of the degree of amphipathic character of a sequence in a given secondary structure (*34*). The amphipathic character of the three α-helical regions in the Antennapedia protein (*36*) is clearly revealed only by the analysis of the combined set of homeodomain sequences. The secondary structure of Arc repressor, a small DNA-binding protein, was recently predicted by a similar method (*8*) and confirmed by nuclear magnetic resonance studies (*37*).

The specific pattern of hydrophobic and hydrophilic residues in an amino acid sequence must limit the number of different structures a given sequence can adopt and may indeed define its overall fold. If this is true, then the arrangement of hydrophobic and hydrophilic residues should be a characteristic feature of a particular fold. Sweet and Eisenberg have shown that the correlation of the pattern of hydrophobicity between two protein sequences is a good criterion for their structural relatedness (*38*). In addition, several studies indicate that patterns of obligatory hydrophobic positions identified from aligned sequences are distinctive features of sequences that adopt the same structure (*4, 29, 38, 39*). Thus, the order of hydrophobic and hydrophilic residues in a sequence may actually be sufficient information to determine the basic folding pattern of a protein sequence.

Although the pattern of sequence hydrophobicity may be a characteristic feature of a particular fold, it is not yet clear how such patterns could be used for prediction of structure de novo. It is important to understand how patterns in sequence space can be related to structures in conformation space. Lau and Dill have approached this problem by studying the properties of simple sequences composed only of H (hydrophobic) and P (polar) groups on two-dimensional lattices (*40*). An example of such a representation is shown in Fig. 5. Residues adjacent in the sequence must occupy adjacent squares on the lattice, and two residues cannot occupy the same space. Free energies of particular conformations are evaluated with a single term, an attraction of H groups. By considering chains of ten residues, an exhaustive conformational search for all 1024 possible sequences of H and P residues was possible. For longer sequences only a representative fraction of the allowed sequence or conformation space could be explored. The significant results were as follows: (i) not all sequences can fold into a "native" structure and only a few sequences form a unique native structure; (ii) the probability that a sequence will adopt a unique native structure increases with chain length; and (iii) the native states are compact, contain a hydrophobic core surrounded by polar residues, and contain significant secondary structure. Although the gap between these two-dimensional simulations and three-dimensional structures is large, the use of simple rules and sequence representations yields results similar to those expected for real proteins. Three-dimensional lattice methods are also beginning to be developed and evaluated (*41*).

Summary

There is more information in a set of related sequences than in a single sequence. A number of practical applications arise from an analysis of the tolerance of residue positions to change. First, such information permits the evaluation of a residue's importance to the function and stability of a protein. This ability to identify the essential elements of a protein sequence may improve our understanding of the determinants of protein folding and stability as well as protein function. Second, patterns of tolerance to amino acid substitutions of varying hydrophilicity can help to identify residues likely to be buried in a protein structure and those likely to occupy

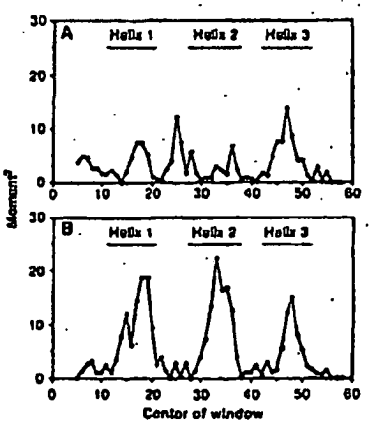


Fig. 4. Helical hydrophobic moments calculated by using (A) the Antennapedia homeodomain sequence or (B) a set of 39 aligned homeodomain sequences (*35*). The bars indicate the extent of the helical regions identified in nuclear magnetic resonance studies of the Antennapedia homeodomain (*36*). To determine hydrophobic moments, residues were assigned to one of three groups: H1 (high hydrophobicity = Trp, Ile, Phe, Leu, Met, Val, or Cys); H2 (medium hydrophobicity = Tyr, Pro, Ala, Thr, His, Gly, or Ser); and H3 (low hydrophobicity = Gln, Asn, Glu, Asp, Lys, or Arg). For the aligned homeodomain sequences, the residues at each position were sorted by their hydrophobicity by using the scale of Fauchere and Pliska (*43*). Arg and Lys were not counted unless no other residue was found at the position, because they contain long aliphatic side chains and can thereby substitute for nonpolar residues at some buried sites. To account for possible sequence errors and rare exceptions, the most hydrophilic residue allowed at each position was discarded unless it was observed twice. The second most hydrophilic residue was then chosen to represent the hydrophobicity of each position. An eight-residue window was used and the vectors projected radially every 100°. The vector magnitudes were assigned a value of 1, 0, or −1 for positions where the hydrophobicity group was H1, H2, or H3, respectively.

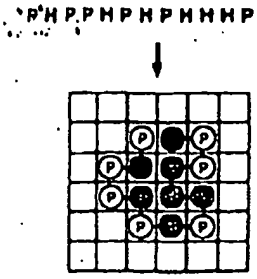


Fig. 5. A representation of one compact conformation for a particular sequence of H and P residues on a two-dimensional square lattice. [Adapted from (*40*), with permission of the American Chemical Society]

surface positions. The amphipathic patterns that emerge can be used to identify probable regions of secondary structure. Third, incorporating a knowledge of allowed substitutions can improve the ability to detect and align distantly related proteins because the essential residues can be given prominence in the alignment scoring.

As more sequences are determined, it becomes increasingly likely that a protein of interest is a member of a family of related sequences. If this is not the case, it is now possible to use genetic methods to generate lists of allowed amino acid substitutions. Consequently, at least in the short term, it may not be necessary to solve the folding problem for individual protein sequences. Instead, information from sequence sets could be used. Perhaps by simplifying sequence space through the identification of key residues, and by simplifying conformation space as in the lattice methods, it will be possible to develop algorithms to generate a limited number of trial structures. These trial structures could then, in turn, be evaluated by further experiments and more sophisticated energy calculations.

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